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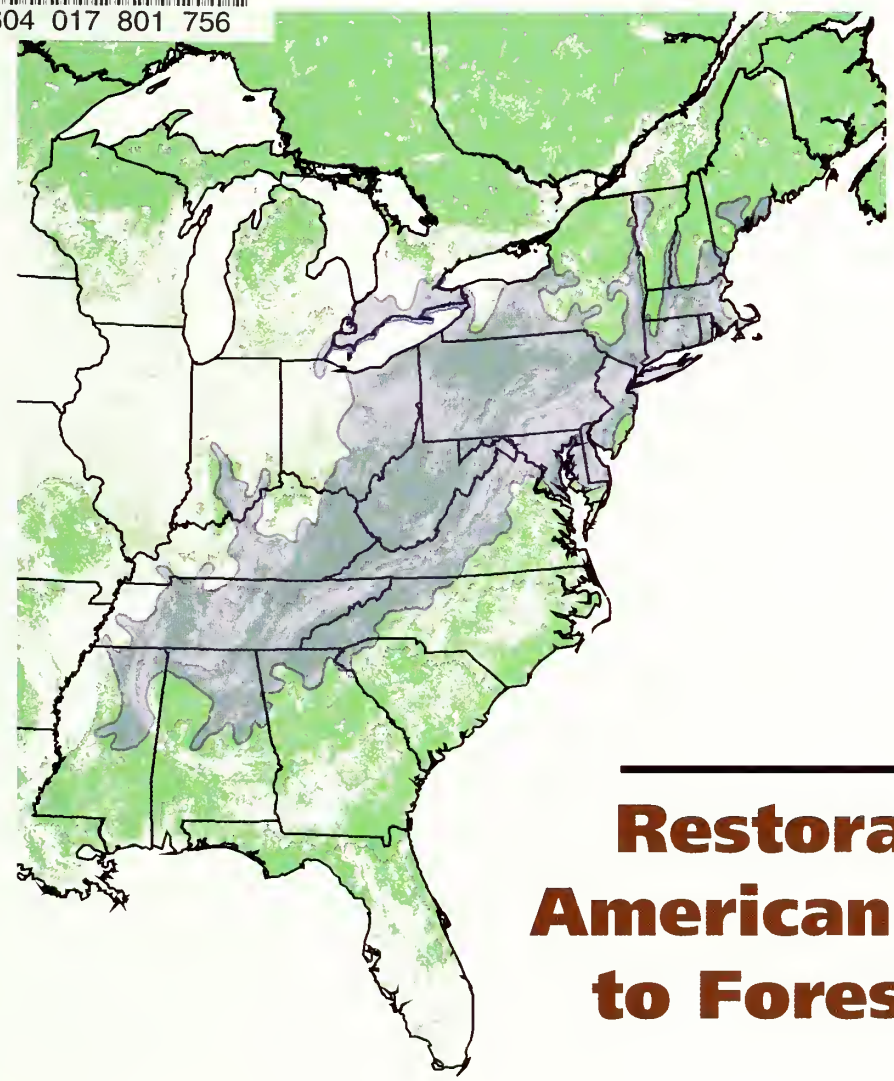

Restoration of American Chestnut to Forest Lands

Proceedings of a Conference and Workshop Held May 4 – 6, 2004 at The North Carolina Arboretum

Edited by Kim C. Steiner
and John E. Carlson

Natural Resources Report NPS/NCR/CUE/NRR — 2006/01

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held at
The North Carolina Arboretum
Asheville, North Carolina, U. S. A.
May 4–6, 2004

Natural Resources Report NPS/NCR/CUE/NRR – 2006/001

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Sponsored by:

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Front Cover:

The figure on the front cover depicts the core of the natural range of American chestnut (as delineated by Elbert Little) superimposed on a map of the present occurrence of forest in the United States (http://nationalatlas.gov/mld/foresti.html) and Canada (http://geogratis.cgdi.gc.ca/clf/en). Small, outlying populations of the original American chestnut range are not shown.

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PREFACE

The papers in this document were presented at a conference and workshop that was held at The North Carolina Arboretum in Asheville, NC, May 4-6, 2004. The purpose of the conference was to discuss issues surrounding the restoration of American chestnut to forest lands. The audience members were primarily employees of USDI National Park Service (NPS), and interest focused on the question of restoring chestnut to NPS lands, but most presentations were selected to address restoration from the broadest perspective possible. The organizers of the conference were Drs. Kim Steiner and John Carlson of the School of Forest Resources at The Pennsylvania State University, and the sponsors were the Chesapeake Watershed Cooperative Ecosystem Studies Unit (CESU) (NPS), the Southern Appalachian Mountains CESU (NPS), and The Pennsylvania State University.

The conference covered the current status of chestnut blight research and objectives, opportunities, and potential directions for American chestnut restoration programs on NPS lands. Topics discussed at the meeting included policy issues, the current status of chestnut, chestnut ecology, breeding programs, blight resistance technologies, genetic issues, potential impacts on forest ecology, design of restoration programs, and knowledge gaps related to restoration within the National Park System. The conference ended with half-day workshop facilitated by Dr. James Finley of the School of Forest Resources at The Pennsylvania State University. Attendees remarked that the scope and quality of presentations established the meeting as a benchmark event in the history of chestnut restoration. As a result of the meeting, a summary of issues and recommendations for National Park Service administrators is being prepared. This collection of papers represents the most comprehensive and current information available at this time on the biology of American chestnut and the blight fungus and the potential for restoring chestnut to its native range.

John E. Carlson and Kim C. Steiner, August 4, 2005

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INTRODUCTION

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The American chestnut (*Castanea dentata* (Marsh.) Borkh.) once accounted for a quarter of the hardwood trees throughout the eastern deciduous forest and in some locations in the southern Appalachians, its density reached 70-85%. A rapid, dramatic decline in the species dominance began around 1900 with the introduction from the Orient of the chestnut blight fungus, *Cryphonectria parasitica* (Murrill) Barr. In spite of early efforts at eradication, within 30 years the fungus had spread throughout the chestnut's entire native range, which extends from Maine to Georgia, Alabama, and Mississippi and west to eastern Michigan and southern Illinois. By 1940 practically all of the chestnuts throughout the range were dead or infected.

As the chestnut declined, the eastern deciduous forest dramatically changed. Other species filled the void, principally oak, hickory, and pine in the southern Appalachians creating the Mixed Oak, Oak-Pine, Mixed Mesophytic, and Oak-Hickory Forest associations and hemlock, sugar maple, and beech to the north in the Allegheny Mountains, which is primarily the Beech-Maple Forest association. While the chestnut has relinquished its dominant role, its legacy continues through its regenerative capacity to resprout from the roots of long infected trees. The American chestnut exists today largely as a clonal understory sapling or pole tree, rarely living longer than 10 to 40 years. However, some of these sprouted trees are able to set fruit before succumbing, but the new seedlings rapidly become infected. The importance of this sexual reproduction cannot be underestimated when thinking in terms of evolutionary time. Someday, long in the future, there may be successful seedling recruitment.

Few ecological disasters have generated as much interest as chestnut blight. Shortly after the disease was first recognized in New York in 1904, research endeavored to understand every aspect of the disease and its exotic causal agent. Over the years much has been learned. In recent decades significant progress has been made in several areas, including the selection and breeding for blight resistance and the discovery and enhancement of fungal hypovirulence. Hypovirulent strains of *C. parasitica* contain infectious cytoplasmic viruses that reduce the ability of the pathogen to cause cankers. Much of this research has captured the interest of the public renewing hope that this iconic American species will eventually reappear in the eastern deciduous forest. The National Park Service, which manages many parks throughout the former chestnut domain, will undoubtedly be expected to engage these advances and fulfill this dream. While these developments may still be several or many years away from practical application, the prognosis for eventually managing chestnut blight is promising. Consequently, the National Park Service must begin to fully understand the promise and pitfalls of these advances and to explore the limitations and consequences of restoration.

This series of presentations and the following discussion are intended to assist the National Park Service in fulfilling three objectives:

Our first objective is to develop a comprehensive understanding of all the science and technology that hold significant promise for restoring the American chestnut. Hopefully, through these presentations we will be able to assess the feasibility and potential for success as well as the long-term consequences these advances could impose on the ecosystem. The significant areas of interest include biocontrol through the use of hypovirulence, the selection and breeding of naturally-occurring putative resistant American chestnuts, back crossing the American chestnut with the resistant Chinese chestnut, and transgenic

approaches to enhance host resistance and to debilitate the pathogen, as well as the potential for combining these technologies into an integrated program.

Our second objective is to define our goals for American chestnut restoration. We recognize that these technologies promise a range of restoration possibilities, from the minimal establishment of demonstration plantings to the incorporation of resistant selections and biocontrol agents into major reforestation projects. While these technological advances are driving our immediate interest in restoration, our decisions must also be guided by an understanding and appreciation of the ecological consequences posed by restoration choices. The National Park Service must have a thorough discussion as to whether our restoration goals and the technologies we select to achieve these goals are compatible with our policies, management objectives and most importantly our resources.

Based on the status and feasibility of the technology and our restoration goals, the third objective is to prescribe how the Service and the parks should proceed. What are the acceptable choices today, what promising technologies will we endorse in the future, what policy issues must we address, what research do we believe is still necessary on unanswered questions or issues, and how can the National Park Service assist? The implementation of some technology, such as the use of transgenic chestnuts or bioengineered hypovirulent strains for biocontrol, may necessitate policy decisions. Decisions relating to transgenic organisms are also relevant to other restoration and management issues affecting the Service and are under discussion now. Other approaches, such as the planting of hybrids may soon be available. However, this option, like all tree planting efforts, has long-term consequences and the decision to proceed should be well founded. We may decide that the research findings are premature or inconclusive, the long-term prospects uncertain, and additional study is necessary before we begin to engage in large scale restoration programs. While understandably, parks may differ in their restoration objectives; their decision process must be consistent and based on the best available science. The visiting public has always been interested in the ecological and cultural heritage of the American chestnut in their National Parks. Consequently, the National Parks will present the most visible and critiqued application of chestnut restoration technology. The public fully and rightfully expects us to understand and support the decisions we make.

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NATIONAL PARK SERVICE MANAGEMENT POLICY GUIDANCE FOR RESTORATION OF AMERICAN CHESTNUT TO NATIONAL PARK SYSTEM UNITS

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Abstract: The National Park Service's Management Policies 2001 provide clear guidance for decisions regarding management of the nearly extirpated American chestnut (*Castanea dentata*). Restoration is appropriate and may involve active planting, cross breeding, and genetic engineering using genotypes from areas within and outside the parks. Restoration must be based on science, analyzed through environmental compliance processes, include the public, and consider actively involving partners.

Keywords: management policies / restoration / American chestnut / blight / exotic species

INTRODUCTION

Law, policy, philosophy, and science contribute to decision-making about whether or not to attempt to restore the American chestnut (*Castanea dentata*) to one or more units of the National Park System. The following discussion addresses these components of decision-making by examining specific provisions and then identifying possible pathways for applying them to management actions.

EXCERPTS FROM NATIONAL PARK SERVICE MANAGEMENT POLICIES 2001

The National Park Service develops and publishes the Management Policies to interpret the many laws that authorize and direct the purposes, uses, and management of lands incorporated into the National Park System and to fill in details not specifically addressed in the laws. The Service's Management Policies 2001 provide both general and specific guidance regarding management of park natural resources. The statements provided in this section are taken directly from, or paraphrase, selected entries in the Management Policies 2001.

Natural Conditions

In its most general terms, the Management Policies 2001 directs the Service to preserve the natural resources, processes, systems, and values of units of the national park system in an unimpaired, evolving condition. More specifically, they direct the Service to:

- preserve the natural resources, processes, systems, and values of units of the national park system in an unimpaired condition, to perpetuate their inherent integrity and to provide present and future generations with the opportunity to enjoy them;
- recognize that natural processes and species are evolving and allow this evolution to continue, minimally influenced by human actions; and
- apply the term "natural condition" to mean the condition of resources that would occur in the absence of human dominance over the landscape.

Natural Ecosystems and Native Species

The Service becomes more specific in its policy guidance for natural ecosystems and native species through four major concepts. The first concept provides a broad ecosystem overview: the Service generally does not intervene in natural biological or physical processes – when it does, such as to remove human-impacts to natural ecosystem functioning, it bases its actions on clearly articulated, well-supported management objectives and the best scientific information available. The following specific statements guide the implementation of this ecosystem approach:

- the Service will not intervene in natural biological or physical processes, with certain exceptions;
- actions to restore natural ecosystem functioning that has been disrupted by past or ongoing human activities will use the minimum necessary interventions to achieve the stated management objectives;
- biological or physical processes altered in the past by human activities may need to be actively managed to restore them to a natural condition or to maintain the closest approximation of the natural condition in situations in which a truly natural system is no longer attainable;
- landscape and vegetation conditions altered by human activity may be manipulated where the park management plan provides for restoring the lands to a natural condition;
- revegetation efforts usually will use propagules representing species and gene pools native to the ecological portion of the park in which the restoration project is occurring but may use improved varieties or closely related native species for a natural area so degraded that restoration with gene pools native to the park has proven unsuccessful;
- because naturally ignited fire is a natural process in many ecosystems sustained in parks, each park with vegetation capable of burning will prepare a fire management plan addressing natural and cultural resource objectives; safety considerations for park visitors, employees, neighbors, and developed facilities; and potential impacts to public and private property adjacent to the park; and
- the extent and degree of management actions taken to protect or restore park ecosystems or their components will be based on clearly articulated, well-supported management objectives and the best scientific information available.

The second major concept addresses species population dynamics: the Service generally does not intervene in native plant and animal species dynamics and natural fluctuations in their populations. Components of this concept include:

- native species are species that have occurred or now occur as a result of natural processes on lands designated as units of the national park system and so native species in a place are evolving in concert with each other;
- natural processes are relied upon to maintain native plant and animal species and to influence natural fluctuations in populations of these species;
- the Service maintains as parts of park natural ecosystems all native plants and animals by:
    - preserving and restoring natural abundances, diversities, dynamics, distributions, habitats, and behaviors of native plant and animal populations and the communities and ecosystems in which they occur;
    - restoring native plant and animal populations in parks when they have been extirpated by past human-caused actions; and
    - minimizing human impacts on native plants, animals, populations, communities, and ecosystems, and the processes that sustain them.

The third major concept addresses protection of the full genotypic range of native plant and animal populations in the parks. Features of this concept include:

- individual plants and animals found within parks are genetically parts of species populations that may extend across both park and non-park lands; providing for the persistence of a species in a park may require maintaining a number of local populations, often both within and outside the park;
- protecting the full range of genetic types (genotypes) of native plant and animal populations in the parks is achieved by perpetuating natural evolutionary processes and minimizing human interference with evolving genetic diversity;
- steps for protecting species native to national park system units that are listed under the Endangered Species Act include:
    - active management programs to inventory, monitor, restore, and maintain habitats of listed species, control detrimental non-native species, control detrimental visitor access, and re-establish extirpated populations as necessary to maintain the species and the habitats upon which they depend;
    - cooperation with other agencies, states, and private entities to promote candidate conservation agreements aimed at precluding the need to list species; and
    - developing management actions for the protection and perpetuation of federally, state, or locally listed species through the park management planning process, including consultation with lead federal and state agencies as appropriate;
- intervention to manage individuals or populations of native species only when:
    - such intervention will not cause unacceptable impacts to the populations of the species or to other components and processes of the ecosystems that support them;
    - management is necessary to protect rare, threatened, or endangered species; or
    - removal of individuals or parts thereof is part of an approved research project; is done to provide propagules for restoring native populations in parks or cooperating areas without diminishing the viability of the park populations from which the individuals are taken; or meets specific park management objectives;
- restoration of extirpated native plant and animal species to parks whenever all of the following criteria are met:
    - adequate habitat to support the species exists or can be restored in the park, and if necessary also on adjacent public lands and waters, and, once a natural population level is achieved, the population can be self-perpetuating;
    - the species does not, based on an effective management plan, pose a serious threat to the safety of people in parks, park resources, or persons or property outside park boundaries;
    - the genetic type used in restoration most nearly approximates the extirpated genetic type; and
    - the species disappeared, or was substantially diminished, as a direct or indirect result of human-induced change to the species population or to the ecosystem;
- the need to maintain appropriate levels of genetic diversity will guide decisions on what actions to take to manage isolated populations of species or to enhance population recovery; and
- actions to transplant organisms for purposes of restoring genetic variability through gene flow between native breeding populations will be preceded by an assessment of the genetic compatibility of the populations.

The fourth major concept addresses methods for obtaining propagules for restoring plant species to parks:

- programs to restore plant species may include propagating plants in greenhouses, gardens, or other confined areas to develop propagules for restoration efforts or to manage a population's gene pool.

Pest Management

The Service provides specific policy guidance regarding pest management. It relies on integrated pest management (IPM - a decision-making process that coordinates knowledge of pest biology, the environment, and available technology to prevent unacceptable levels of pest damage, by cost-effective means, while posing the least possible risk to people, resources, and the environment) to guide managing pests in parks. It monitors use of pesticides (any substance or mixture that is used in any manner to destroy, repel, or control the growth of any viral, microbial, plant, or animal pest) in parks through case-by-case review of pesticide use requests, taking into account environmental effects, cost and staffing, and other relevant considerations. It allows use of a chemical, biological, or bio-engineered pesticide in a management strategy following a determination by a designated IPM specialist that such use is necessary, and that all other available options are either not acceptable or not feasible.

Managing Non-Native Species

The Service identifies as exotic (non-native, alien, or invasive) species those species that occupy or could occupy park lands directly or indirectly as the result of deliberate or accidental human activities. The Service devotes significant management attention to exotic species because these are species that did not evolve in concert with the species native to the place, are not a natural component of the natural ecosystem at that place, and, as a result, threaten the naturalness of the ecosystem being preserved to the degree that they out-compete the native species or alter the natural processes of the ecosystem.

The Service's goal for managing exotic species is to not allow them to displace native species if displacement can be prevented. In general, new exotic species will not be introduced into natural ecosystems in parks while, in rare situations, an exotic species may be introduced or maintained to meet specific, identified management needs when all feasible and prudent measures to minimize the risk of harm have been taken. Such deliberate introductions may occur when the species is:

- a closely related race, subspecies, or hybrid of an extirpated native species; or
- an improved variety of a native species in situations in which the natural variety cannot survive current, human-altered environmental conditions; or
- used to control another, already- established exotic species.

In some situations, exotic plant and animal species are maintained to meet an identified park purpose. In all other situations, exotic plant and animal species that do not meet an identified park purpose will be managed— up to and including eradication— if (1) control is prudent and feasible, and (2) the exotic species:

- interferes with natural processes and the perpetuation of natural features, native species or natural habitats; or
- disrupts the genetic integrity of native species; or
- disrupts the accurate presentation of a cultural landscape; or
- damages cultural resources; or
- significantly hampers the management of park or adjacent lands.

For species requiring management, high priority will be given to managing those exotic species that have, or potentially could have, a substantial impact on park resources, and that can reasonably be expected to be successfully controlled. Lower priority will be given to exotic species that have almost no impact on park resources or that probably cannot be successfully controlled.

The decision to initiate management is based on a determination that the species is exotic. For species determined to be exotic and where management appears to be feasible and effective, parks evaluate the species' current or potential impact on park resources; develop and implement exotic species management plans according to established planning procedures; consult, as appropriate, with federal and state agencies; and invite public review and comment, where appropriate. In designing programs to manage exotic species, parks seek to avoid causing significant damage to native species, natural ecological communities, natural ecological processes, cultural resources, and human health and safety.

Soil Management

Any program to restore plants to natural systems must recognize and provide for soil management to the degree that the natural soil condition has been disrupted by past human activities. As a result, the Service seeks to understand and preserve the soil resources of parks, and to prevent, to the extent possible, the unnatural erosion, physical removal, or contamination of the soil, or its contamination of other resources. Where necessary, the Service may import off-site soil or soil amendments to restore damaged sites where such use of a soil, fertilizer, or other soil amendment may be appropriate, provided that the use does not unacceptably alter the physical, chemical, or biological characteristics of the soil, biological community, or surface or ground waters. Soil obtained from off-site normally will be salvaged soil, not soil removed from pristine sites, unless the use of pristine site soil can be achieved without causing any overall ecosystem impairment to the donor site.

PHILOSOPHICAL FOUNDATION

The conceptual goal of seeking to manage parks to achieve the natural condition as defined in the Management Policies clearly is impractical to achieve given the already existing degree of human dominance over the entire earth. However, identifying the natural condition as the desired condition is useful. With respect to the question of restoring the chestnut to parks, such identification gives park managers a clearly stated, measurable goal towards which to direct their scientific study and resource management efforts. Park managers address this goal by developing achievable intermediate goals and practical steps for achieving those intermediate goals. The remainder of this paper focuses on practical, policy-appropriate science and management steps that warrant consideration in efforts to develop a chestnut restoration plan and supporting program.

APPLICATION OF NPS MANAGEMENT POLICIES TO RESTORATION OF CHESTNUT TO PARKS

Role of Science

Decisions about natural resource management are based on planning supported by scientific and scholarly information, environmental evaluation, and public involvement. Scientific activities of inventory, monitoring, research, and assessment are important components of a chestnut restoration program because they:

- contribute to developing a long-range strategy;
- guide the functioning of interdisciplinary teams and processes;
- permit articulating the desired future conditions for the park's natural resources;
- provide the tools for obtaining and integrating the best available science;
- generate understanding of the effects of management actions on natural resources whose function and significance are not clearly understood;
- provide the framework for applying long-term research or monitoring in an adaptive management context to evaluate results;
- provide the data for fully and openly evaluating environmental costs and benefits and, through public involvement, incorporating mitigation measures; and
- underlie planning for clearly avoiding impairment of park natural resources and values.

Potential Role of Special Designation Areas

The Management Policies make available to parks two special site designations (Research Natural Areas and Experimental Research Areas) that could be used to facilitate and focus efforts to restore the chestnut to parks. Research Natural Areas are sites within parks that contain prime examples of natural resources and processes, including significant genetic resources, and that have value for long-term observational studies or as control areas for manipulative research taking place outside the parks. Experimental Research Areas are specific tracts in limited situations that are managed for approved manipulative research, which is research involving conscious alteration of existing conditions as part of the experimental design.

Activities in Research Natural Areas generally will be restricted to non-manipulative research, education, and other activities that will not detract from an area's research values. Activities in Experimental Research Areas involve a greater degree of manipulation as part of the research design but also can include other potential uses, such as education or other activities that will not detract from the area's research purpose.

Decisions and Actions Involve Partners

NPS fully recognizes that many organizations are involved in efforts to restore the chestnut to the forests of the United States. Management Policies encourage park managers to develop agreements appropriately with others to coordinate chestnut restoration activities in ways that would maintain and protect, not compromise, park resources and values, including the integrity of native gene pools and natural ranges of species and ecological communities. In entering into such agreements, park managers would be encouraged to work with other land managers to encourage the conservation of populations and habitats of the chestnut wherever and whenever possible, including through such NPS actions as:

- participating in local and regional scientific and planning efforts, identifying chestnut local population characteristics and ranges, and developing cooperative strategies for maintaining or restoring park components of these local populations;
- preventing the introduction of new exotic species into units of the National Park System while removing populations of the chestnut blight that have already become established in parks, and
- providing small quantities of chestnut genetic material from parks for cooperators to use in selective breeding, genetic engineering, or propagule generation efforts.

At the same time, the Policies encourage managers to avoid the dissemination into the wild of chestnut genetic material outside the native range of the chestnut, unless such dissemination is conducted under a

specific, scientifically-based management program designed to mitigate for a human-facilitated environmental impact, such as habitat fragmentation or global climate change.

Decisions and Actions to Restore the Chestnut

Park actions to restore the chestnut would respond to clear goals, implement a proactive strategy, and be based on the clear responsibility park managers have to preserve natural conditions. Goals could include:

- re-establishing in human-disturbed components of park natural systems (those where introduction of the chestnut blight has nearly eliminated a dominant native species) the natural functions and processes provided by the chestnut by restoring appropriate chestnut genotypes or the best available surrogates;
- using the best available technology, within available resources, to restore the chestnut and, as a result, to stimulate restoration of its associated biological and physical system components and accelerate recovery of landscape and biological-community structure and function; and
- removing the exotic species or at least greatly reducing its role in the ecosystem.

Elements of an appropriate strategy would include:

- maintaining existing, in-park, local populations of native genotype plants that continue to resprout following blight-induced death of their previous sprouts for the purpose of maintaining living genetic material for future research efforts
- restoring the native species using organisms taken from populations as closely related genetically and ecologically as possible to park populations, preferably from similar habitats in adjacent or local areas, where possible;
- introducing different native genotypes where the management goal is to increase the variability of the park gene pool to mitigate past, human-induced loss of genetic variability; guided by knowledge of local adaptations, ranges, and habitat requirements, and detailed knowledge of site ecological histories;
- introducing novel, non-native genotypes where the management goal is to develop a gene pool that is genetically resistant to the chestnut blight, guided by a goal of inserting the resistance with as minimal an insertion of other non-native genes as possible;
- applying the Service's integrated pest management (IPM) program to eradicate, or at least control, the chestnut blight to whatever degree possible while reducing risks to the public, park resources, and the environment from chestnut blight and blight management strategies; and
- utilizing appropriate soil conservation and soil amendment practices to facilitate restoring the chestnut in ways that prevent or minimize adverse, potentially irreversible impacts on soils.

Given that the park is the basis and focus of NPS natural resource management programs, it is important to recognize that:

- resource management is a local activity and the park superintendent exercises the responsibility for, and is held accountable for, all actions that occur within the park – therefore, cooperative actions to develop an NPS chestnut restoration activity would use the park and its partners as the basic building block; and
- the Service's use of networks of parks for inventory and monitoring purposes would offer a strategic opportunity for cooperatively applying metapopulation and biological corridor concepts to chestnut restoration efforts.

DISCUSSION

From this review of NPS Management Policies, it becomes clear that NPS policy is not an issue for determining whether or not to restore the chestnut to parks. Our current state of knowledge about the status of the chestnut meets key policy provisions:

- extirpation is occurring and its cause is known to be an exotic species, hence the extirpation is human-caused and management restoration is appropriate;
- the impact of the extirpation on park natural resources is apparent – loss of a dominant species, probability of cascading ecological effects, associated human social and economic effects, all of which possibly may constitute impairment; and
- a management response is clearly possible – minimize the effect of the exotic species both by controlling the exotic species and by developing and planting seeds, seedlings, and saplings of a blight-resistant chestnut genotype.

These policy provisions suggest a clear goal - restore a naturally functioning, natural ecosystem by restoring a nearly extirpated native species, eliminating the exotic species or at least neutralizing its impact on that native species, and restoring the ecosystem function once provided by the native species.

The policy goal of maintaining a practically appropriate level of genetic fidelity can be met. First, although many of the original local population gene pools are so diminished they probably can not be restored, there are a few existing, endemic, apparently disease-resistant North American genotypes that can be propagated and disseminated as a means of maintaining at least some native genetic material in the gene pools used for restoration. Second, specific, appropriate genes from several nearest-relative gene pools can be injected into the residually available North American gene pools either through cross breeding of North American and Asian genotypes or through using genetic engineering to insert selected foreign genes into the residual native genotypes.

If a decision were to be made to implement this policy of restoring a species and its associated ecosystem, there are clear science needs that must be met as part of planning, NEPA analysis, and developing restoration methods. These information needs include:

- understanding how the existing ecosystems and their current floras, faunas, and physical features might change if restoration were successful;
- assessing whether any native species would become at risk if restoration were successful;
- determining if there would be any risk of introducing other pathogens in association with planting cultivated seeds or young trees;
- addressing what side effects, if any, individual park ecosystems or their local chestnut populations would experience as a result of addition of a genetically modified chestnut to the individual ecosystems;
- determining whether any physical alteration of existing ecosystems would be needed to achieve an effective restoration and, if so, what would those alterations be; and
- developing park chestnut restoration activities as scientific experiments with good design, methods, replication, and documentation – in essence, structuring these activities as adaptive management.

Carrying out a chestnut restoration program clearly would have to involve the public. The program would be a long term activity requiring support over many years. It would depend on the involvement and good will of many partners. It would have to be based on a clear understanding by all participants of the scientific basis for, and methodological requirements of, each of the possible management approaches.

Because of its ultimate wide spread distribution over the landscape and through time, its probability of success would depend on the level of stakeholder agreement, support, and participation maintained across space and through time.

The NPS Management Policies provide a framework for determining what kinds of restoration management action might be appropriate, for ensuring that scientific findings play a significant role in informing the determination, and for broadly and effectively involving the public in the decision-making process. For whatever management program might be adopted, the Policies leave to the discretion of the site manager what specific mix of technologies to apply, with the mix at any given site being influenced by such site-specific factors as what the science shows to be technically possible, what the environmental analysis shows to be the trade-offs between environmental and human benefit and detrimental impact, what actions the public involvement reveals to be locally and generally acceptable, and what fiscal and human resources are likely to be available for conducting the management program over the projected duration of the restoration effort.

CONCLUSION

National Park Service management policies encourage restoration of the chestnut to park ecosystems. These policies require that such restoration be accomplished using a process that includes science, planning, and public involvement. These policies strongly encourage adopting a management program that emphasizes cooperation and collaboration with partners.

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HISTORICAL ECOLOGY OF AMERICAN CHESTNUT (*CASTANEA DENTATA*)

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Abstract: American chestnut (*Castanea dentata*) was a very common species in the forests of eastern North America in the early 20th century when it was decimated by the introduced chestnut blight. The post-glacial migration history of chestnut differed from its most common associates, oak and hickory due most likely to differences in the ecological tolerances of the species. By 1500, both pollen evidence and historical documents indicate that chestnut trees formed 5-15% of oak-dominated forests throughout the northeast, as far north as southern New England. The vigorous sprouting of chestnut after it is cut allowed it to develop widespread "sprout forests," where chestnut trees were 50% or more of the stems in many stands, after 18th-19th century logging. This high concentration of chestnut stems may have allowed the blight to spread very quickly throughout its range. In addition, many other changes have occurred in regional forests over the last century, as they have responded to a variety of human-caused disturbances. Thus, introduction attempts should take into account the time period that is of interest to try to restore and the dynamics of current forests in considering what might be the fate of chestnut trees that they reintroduce into today's forests.

Key Words: historical ecology / sprout forests / pollen / historical documents

INTRODUCTION

Doomed sprouts of American chestnut (*Castanea dentata*) are widely dispersed throughout the hardwood and hemlock/hardwood forests of the eastern United States today. In 1900, mature chestnut trees dominated many of these stands, and were very common in many others. A species valued not only for its majestic beauty, but also for timber and fruit, chestnut trees were planted well beyond their natural range. A deadly chestnut blight from an introduced tree in New York City, however, destroyed all mature trees in the early 20th century, leaving only ghostly stumps and shrubby sprouts as legacies of this once majestic tree.

A brighter future for the American chestnut may now be in the hands of foresters who have developed strains resistant to the blight. In considering the possibility of restoring this native tree to its former habitats, it is important to consider its former range, and its unique history as it developed to its distribution at the time the blight killed the trees. There are two major reasons to consider this history.

- First, we need to establish an appropriate goal for restoration. Is there a specific period in the history of this species that is particularly desirable? History can provide analogues that may be considered as possible goals of restoration. This gives the restoration ecologists clear endpoints to consider.

- Second, history can provide a picture of the changing distribution of the species, and of other species that were associated with it, over time, as they may both facilitate and constrain the likely outcome of restoration. Forests are always dynamic in their composition and structure, and understanding these dynamics can allow restoration ecologists to evaluate potential trends and the future of current changes. (Russell 1997).

I will discuss the history of the changing range of American chestnut and the other species with which it was associated in two time periods: 1. from 10,000 BP to 1500 AD, as it migrated to its pre-Columbian

range: 2. from 1500 AD to 1900 AD, after European settlement of the area. This discussion will be based on the record left by trees in the form of pollen preserved in lake sediments and on the historical documentary record. My analysis will focus mainly on the area from northern New Jersey to northern New England, as that is where the most records of the historical distribution have been compiled.

METHODS

Pollen

Pollen preserved in lake sediments provides a unique record of the history of plants, especially in areas which were covered with glaciers in the most recent (Wisconsinan) ice age, where disrupted drainage has left many sedimentary basins which have accumulated sediments over the millennia. Many tree genera, such as pines (*Pinus*) and oaks (*Quercus*), are pollinated by wind, so produce copious amounts of airborne pollen. Others, such as maples (*Acer*), are pollinated by insects, and produce less pollen, since pollination is more assured when the insect carries pollen from tree to tree. American chestnut seems to fall in an intermediate category of pollen production and dispersal. While insects do visit the male catkins of chestnut trees, the trees produce large amounts of windblown pollen.

Pollen is identifiable under the microscope to varying degrees of specificity. Pollen analysts can distinguish oak and hickory pollen only to genus. Chestnut is also identifiable only to genus. However, only two species are found in eastern North America, and of these only *Castanea dentata* is widespread and exists as a large tree that would distribute pollen any distance from the tree that produced it (Paillet 2000).

After pollen is released by a tree, it is carried by the air before falling to the ground. Even wind-pollinated species drop a large proportion of their pollen within a few hundred meters of the tree. After pollen grains land on a body of water, they eventually sink to the bottom and are incorporated into the sediments. As sediments build up over the years, they thus contain a record of the trees and other plants that have grown in the vicinity as well as of the openness of the vegetation. Pollen grains are very resistant to decay in such as situation, and can provide a proxy for reconstructing past plant, especially tree, distributions.

Large bodies of water, greater than a hectare or so, collect windblown pollen from large regions, 10's of kilometers from the lake, because there is a large ratio of surface area/shoreline. Smaller ponds and hollows, on the other hand, may collect pollen from mainly local sources, 50 m or so from the sedimentation site. These differences allow us to reconstruct vegetation that has produced the pollen on both a regional and a very local, stand-level, basis.

When pollen is produced before leaves, as in the oaks, the wind currents often carry the pollen many kilometers. Chestnut trees release their pollen after the leaves have expanded, which means that the pollen, though copious, is often caught by leaves, and does not enter the air currents. This allows us to interpret chestnut distribution from pollen preserved in sediments on a finer scale that we can determine for many other species which produce large amounts of pollen. Finally, pollen of plants that grow close to the ground in a forested landscape is not carried far, and mostly falls directly to the ground. If the trees are cut, however, pollen produced below a meter by plants such as grasses (Poaceae) and ragweed (*Ambrosia*) can be carried several kilometers. The recent ecological history of eastern North America, characterized by massive regional deforestation after the arrival of European settlers can be dated by increases in these weedy species, even when looking at areas that were not locally deforested.

The pollen data that I will discuss come from three sources:

- First, for the brief discussion of the millennial record, I will use data compiled by Thompson Webb III, and published in numerous publications for interpreting many aspects of post-glacial vegetation and climate trends. Specific references to the pollen collections can be found in Bernabo and Webb (1977).

- The second set of data are those compiled by Russell and Davis (Russell et al 1993, Russell and Davis 2001), which include more detailed records of species distributions over the last 500 years, focusing on human impact on species distributions. These data only cover the area from northern New Jersey north to north-central Maine – the area covered by Wisconsinan glaciation. The trends detected in these data are, however, most likely similar to trends farther south, though further study may either confirm or refute that speculation.

- Finally, I will discuss data from some very small sites in central Massachusetts, which allow the reconstruction of very detailed stand histories (Foster and Aber 2004).

Historical Documents

After the arrival of European settlers in North America, written documents serve also to trace the history of the distribution of forest species. Early travelers provided qualitative descriptions of forest resources, often with excellent taxonomic accuracy. These almost always, however, lack any quantitative information. The earliest quantitative data come from land surveys, generally what are referred to as "metes and bounds" surveys which delineate properties. Surveyors were trained to recognize local tree species, and often used trees as markers for property boundary corners. The parts of the United States settled after the American Revolution were surveyed according to a very clearly codified rectilinear survey, but the colonial lands were surveyed by a variety of methods, some quite systematic and others much less so. By assembling these data, we can, however, obtain a remarkably consistent record of the species distributions in the precolonial forests, before settlers cleared them for farms (Loeb 1987, Whitney 1994, Russell 1997, Cogbill 2000).

The second set of documents that can provide evidence for the preblight distribution of American chestnut is the plethora of forest surveys around the end of the 19th century by state surveyors. The states had begun to realize that careless logging, grazing and fires had severely damaged their forest resources. To evaluate the problem, they embarked on systematic surveys to provide information that could guide their efforts to protect and improve their forests. These provide a snapshot of the condition, composition and structure of the forests of this period, when most heavy logging had moved away from the original 13 colonies, leaving regenerating, generally young forests in the east, especially the northeast (Russell 1987).

DISCUSSION

From the end of the Wisconsinan glaciation to 1500 AD

When the extreme cold of the Wisconsinan glaciation dominated the northern half of North America, tree species that today characterize forests north of the terminal moraine ranged far to the south, where climates were considerably colder than they are today. They were found in novel assemblages, depending on the local climate and the ecological tolerances of species for these conditions (Webb1988, Delcourt and Delcourt 1987). The sketchy pollen record from this time period indicates that American chestnut was a fairly minor component of forests dominated by oak, along with some other associates such as hemlock (*Tsuga canadensis*)or black gum (*Nyssa sylvatica*) (Barclay 1957, Bender et al. 1979, Craig

1969) in the southeastern Appalachian region. In Horse Cove Bog, western North Carolina, however, it was represented in quantities of pollen almost equal to oak between about 1400-150 BP (H.R.Delcourt and P.A.Delcourt, Pers. Comm, University of Tennessee, 1996). As climate moderated, the range of chestnut expanded slowly northeastward along the Appalachian and Ridge and Valley Provinces, reaching very large concentrations in some places before again declining (Barclay 1957, Davis 1983, Webb 1988).

The spread of chestnut into the northern forests lagged behind the oaks and hickory (*Carya*). For example, in southern New York oak had reached its current importance in the pollen record about 9000 YBP, while chestnut did not appear above 1% or so until about 4000 YBP (Maenza-Gmelch 1997). Likely explanations include different climate tolerances coupled with its more demanding pollination mechanism. Because American chestnut cannot self pollinate, a single tree growing beyond the current range of the species could not produce fertile seeds to spread from this point, while a hickory or oak tree could do so.

It is also possible that this distinct history is an artifact of studying all species of oak at one time, because they cannot be distinguished in the pollen record. The different species of oak represented in the east have quite varied ecological tolerances, while we can assume that we are tracing just one species in the case of chestnut. After about 2500-2000 BP, chestnut reached its current range. There is some recent evidence based on lake levels correlated with pollen data that it spread north as climate became more humid after 2000 BP (Shuman et al. 2004).

1500 AD to the present

By 1500 AD, chestnut was a consistent member of the oak-dominated forests of many eastern forests, according to the pollen record (Russell et al 1993, Davis 1983, Webb 1988). It has a much more restricted range than oak or hickory throughout its postglacial history, being restricted to a fairly narrow band along the Appalachian physiographic province (Davis 1983). Again, this may in part be due to comparing all the species in one genus to one species. By 1500 AD, chestnut contributed 4-9% of the pollen in lake sediments south of northern Massachusetts, where oak and hickory were the dominant taxa in the forests. North of about 43°N, where spruce (*Picea*), pine, birch (*Betula*), hemlock and beech (*Fagus grandifolia*) dominated the forests, chestnut was generally less than one percent of the pollen indicating that it was at most a minor component of the forests (Russell and Davis 2001).
Historical records confirm and expand upon these pollen data. According to data compiled by G. Gordon Whitney, American chestnut trees were generally 5-15% of the trees listed in early land surveys in Pennsylvania, eastern Ohio, northern New Jersey, extreme southeastern New York, Long Island, Connecticut, Rhode Island and the Connecticut River valley in Massachusetts. White oak (*Quercus alba*) dominated these forests, with 25-65% of the stems, along with 5-15% hickory. These data have not yet been compiled from farther south in the range of the species.

A breakdown of the data from the area from northern New Jersey and to western Massachusetts shows some details of this distribution (Table 1). Chestnut was most common in forests dominated by oak, with little hemlock or beech, while it was less common (though occasionally present) in areas where beech and hemlock dominated the forests. In eastern West Virginia, chestnut was most common on ridgetops, where it formed 15% of witness trees, compared with 2-5% in other topographic positions (Abrams and McCay 1996). In Pennsylvania, chestnut was most common in the Allegheny Mountain physiographic province, though present throughout the state. Here, too, it was most common on hilltops (Abrams and Ruffner 1995).

Table 1. Percent of trees in precolonial land surveys in northern New Jersey, eastern Pennsylvania, eastern New York and western Massachusetts (data from Russell 1981, Bürgi et al. 2000, McIntosh 1962 and unpublished data for the Shawangunk Mts. and Rensselaerville, NY)

	n.e. NJ (Morris Co.)	n.e. PA (Pike Co.)	n.e. PA (Wayne Co.)	e. NY (Shawangunk Mts.)	e. NY (Catskill Mts.)	e. NY (Rensselaerville)	w. MA (Berkshire Co.)
Chestnut	15	7	1	7	0	0	6
Oak	64	40	6	41	0	4	16
Beech	1	3	36	1	50	48	23
Hemlock	0	5	22	4	20	14	19
Maple	4	7	16	6	14	14	11
Pine	0	27	3	6	0	1	7
Total number of trees	199	1921	939	342	3744	114	1730

Some details of local distributions and response to disturbances have been found in studies in central Massachusetts (Foster and Aber 2004). In these studies, pollen from small hollows or mor humus soils reflects the proportion of trees growing within 50 meters or so of the sampling point. Chestnut importance appears to have alternated with oak where oak was dominant. In another site, it appears that chestnut responded quickly to disturbance, but was supplanted by hemlock after the chestnut blight.

Whatever further study may reveal, however, it appears that on a broad scale, of a county or more, chestnut was a consistent but fairly minor associate of oak, especially white oak in the precolonial forests. How can we reconcile this with evidence of forests dominated by chestnut at the turn of the 19th century (Russell 1987)? The answer lies in the physiology of the species, in particular, its tendency to sprout vigorously from the root crown when it is cut (Paillet 2000).

Between the first settlement of the eastern United States by European settlers and 1900, the new inhabitants cleared all but the most remote and difficult to reach forests. Some land was turned into farms, but much that was not good agricultural land was repeatedly cut over for fuel and timber, especially for making charcoal to feed iron forges and furnaces. The forests of the first half of the 20th century were designated by E. Lucy Braun as "sprout hardwoods" referring to this tendency to sprout (Braun 1950). There is evidence in the pollen record for this change in the importance of chestnut in the forests of the northeast (Russell et al 1993, Russell and Davis 2001). Chestnut is one of the species that consistently increases in proportion of the tree pollen after the increase in agricultural indicators in the pollen record. It is not a major pollen producer like birch, which also increased, so the apparently small increase recorded in the pollen most likely translates into a much greater increase in the proportion of trees in the forest.

It is likely, therefore, that the forests that the blight decimated were primed to spread a pest like this. While not forming a monoculture, the species was very common by this time, and thus allowed the blight to spread quickly throughout its range (Russell et al. 1993). The distribution and abundance of sprouts in forests today reflect a forest greatly modified by the impact of European settlers. That these sprouts represent seedling trees, not the forest giants, is even more suggestive of the dynamic position of chestnut in these early 20th century forests (Paillet 2000).

Today's forests reflect this complex history. Chestnut sprouts abound, and their distribution indicates the sites that are most appropriate for chestnut to succeed. Disturbance is a positive force for chestnut growth. The current forests of the United States have changed significantly in species composition in the last 500 years, with a general decrease in the amounts of hemlock and beech and an increase in birch. Given the associates of chestnut in the historical record and its responses to disturbance, it seems likely that it would respond well to current conditions.

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FOREST HEALTH IMPACTS OF THE LOSS OF AMERICAN CHESTNUT
(transcript of presentation)

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INTRODUCTION

I consider thus meeting and these topics – the restoration of American chestnut and trying to return chestnut to an important ecosystem component in southern Appalachia or in the Appalachians of the Eastern United States – to be very important. My life has been consumed recently with sudden oak death. I leave tomorrow to train the last of three groups of people involved in an expanded early detection survey for sudden oak death. I open that training by helping people visualize what it might have been like in the days, weeks and months after the initial discovery of chestnut blight in the Bronx Zoo and it kind of brings the message home. We don't know if that is what is going to happen with sudden oak death, but it does represent one of the possible outcomes on a continuum from innocuous or no impact to a chestnut blight type of scenario.

FOREST HEALTH

> "Health and integrity are not inherent properties of an ecosystem and are not supported by either empirical evidence or ecological theory." (Wicklum and Davies 1995)

I will start with a bit of discussion on forest health. This would be a short talk if this was the definition that we accepted – that essentially there is no such thing as forest health. Health and integrity are not inherent properties of an ecosystem and are not supported by either empirical evidence or ecological theory. I could stop there, but I won't.

A draft Forest Service Policy was set forth in 1996 and '97. It is still a draft because when you try to bring diverse groups of people together and come to consensus, you end up with chaos, usually. This is still a draft policy, but there are elements that I want you to think about as we walk through the talk.

Forest health is measured at a landscape scale. We are not talking about tree health or even stand health, but forest health – consider it on a landscape scale. The notion of forest health carries with it the idea of ecological integrity and that forest components and relationships are all present, functioning, and self-renewing. And you can imagine what the elimination of chestnut as a functioning ecosystem component did in the early part of the 20th century – how was that affected, that ecological integrity component? Forest health also has a human dimension – the idea that forests should provide for human values, uses, products, and services. And those values etc. are fluid; they change with our ideas about why forests are important.

It's appropriate that the previous presentation was about forest history and what was originally here. Ten thousand years ago the forest composition was quite different from what it is today. That pushes back the perspective from what is was like at European settlement and when the first native people were in this area to the idea that these forests are nothing if not ever-changing as a result of the way that people interact with the forest. I will start with this supposition that southern Appalachian forest landscapes are

unprecedented in history. There's never been anything like what you see here today. And the forest that will result in decades hence from what is there today will be like nothing else that has ever existed in the past. The components of these ecosystems were already in place, I've read, about 58 million years ago, ebbing and flowing with ice sheets and fire. But it really wasn't until the last 10,000 years or so, or maybe even more recently than that, that we've had forests that resemble in structure and composition what was present at European settlement. So why has there never been anything like what we see here today? Of course one important thing, and perhaps the most significant element, was the introduction of the chestnut blight, with ground zero at the Bronx Zoo in 1904.

PROGRESSION OF THE CHESTNUT BLIGHT

It may be something of a fallacy to think of the chestnut blight moving through the eastern hardwood forests in a wave, nothing in front of this wave and devastation behind it. But I draw your attention to a little spot of infection in Bedford County, Virginia (circle in Figure 1), four or five years after discovery of the blight in New York, well in front of the general advance. There is no way that occurred from a continuous spread, and I suggest that this and many other infection sites were the result of subsequent introductions or movement of infected material either prior to or after the discovery in New York.

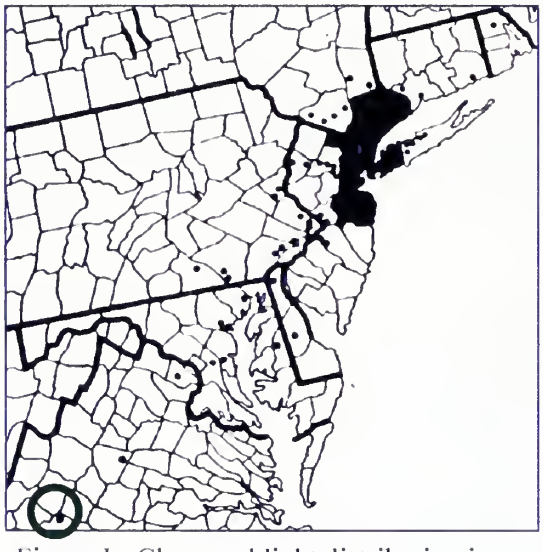

Figure 1. Chestnut blight distribution in 1909 (Metcalf and Collins 1909). O = Bedford County, VA.

In 1911, the infestation in Bedford County, Virginia, was well ahead, or outside of, what might be referred to as the advancing front (Figure 2). Maps from literature published in the 1920's about the progress of the blight through the southern Appalachian assessment area show infection in Greenville County, South Carolina/Henderson County North Carolina, in 1926 (Figure 3). It was known that at the border between Polk, which is the county immediately to the East of Henderson and Greenville County, there was an infestation dated back to 1912, based on the regular increments and dating of cankers at that location. So, there in 1912, and in 1908 in Bedford County, shows that it was not a continuous spread, not an even wave running through the system.

The blight wasn't the only thing going on (in the woods) at that time. There was heavy duty forest utilization. What I try to point out to people, is that what forms the structure of today's forest is a result of not just the chestnut blight, because land use practices and events immediately prior to and just after the chestnut blight were very important as well. Some of those were fire and heavy utilization, and then with regard to fire, not just the presence of fire, but then the almost complete absence of fire following the Weeks Act and the formation of the National Forest and Cooperative Forest Fire Control Programs in the states. So you went from a heavy disturbance regime, introducing chestnut blight on top of that, and then ceasing most heavy disturbance activities.

Table 1 summarizes the pre-1900 and current conditions of the southern Appalachian forests. The southern Appalachian forests before 1900 were dominated by American chestnut in many places. Whether this was an artifact of disturbance by native people or early European activities is less relevant than what was there and being impacted at the time. But anywhere from a quarter to a third, depending on the inventory that you read from the period, in this core Appalachian area of North Carolina-Tennessee-North Georgia-Virginia, had sparse understories, large, widely spaced overstories, and a high

level of disturbance from farming, logging, and fire. When fire regimes were altered, and with oaks already an important part of the forest, oaks were positioned to take the newly available space that was made available with the loss of chestnuts. So now we have dense understories, dense overstories of somewhat smaller diameter trees, and relatively low disturbance regime as compared with the historical past. And then there was the introduction of the gypsy moth, a non-native defoliator, fires suppression programs and a growing human population. These are the backdrops against which we interpret forest health changes.

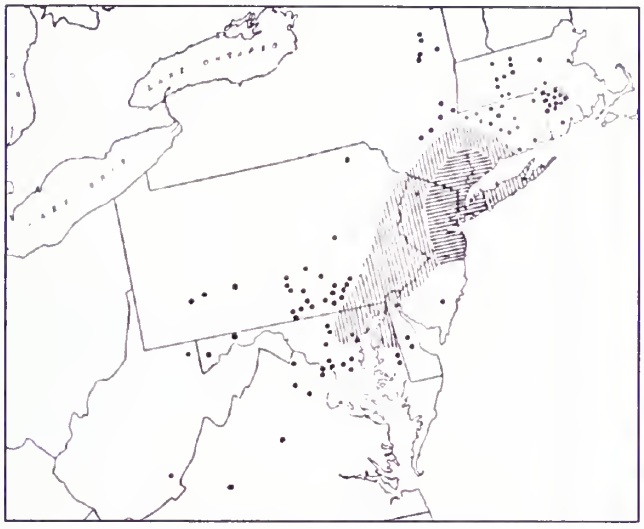

Figure 2. Chestnut blight distribution in 1911 (Metcalf 1912).

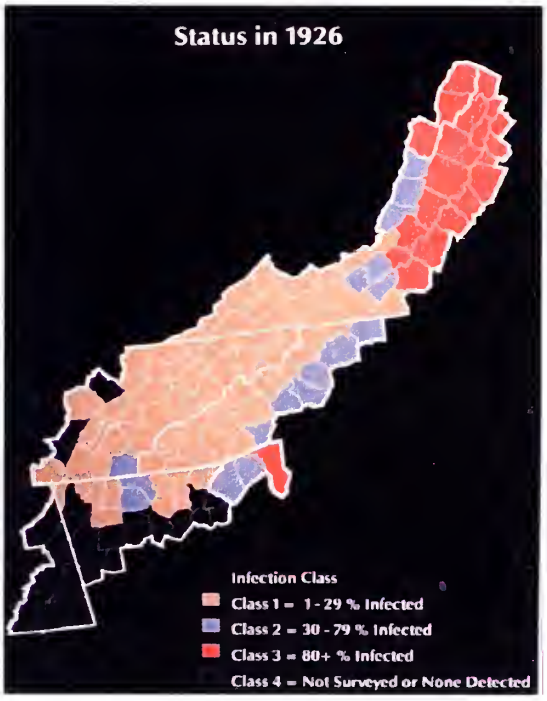

Figure 3. Chestnut blight epidemic in the southern Appalachians in 1926.

Table 1. Composition, structure, and disturbance profiles of southern Appalachian forests, pre 1900's vs. present day.

Pre-1900	**Current**
American chestnut	Aging oak cohort
Sparse understory	Dense understory
Large overstory	Dense overstory
High disturbance	Low disturbance
Farming	Gypsy moth
Logging	Fire suppression
Fire	Human population

This is a quote from Smith (1976) from the 'Changes in Eastern Forests' article in *Perspectives in Forest Entomology*:

> *"We are perhaps entitled to speculate that our chronic and alarming problems with the gypsy moth and other oak defoliators in the eastern or Appalachian portions of the mixed deciduous forest could be as evil a consequence of the chestnut blight as the loss of chestnut itself."*

Oak decline is a disease that I will be discussing as a major forest impact of chestnut blight. Again, these oaks came in as a relatively even-age cohort after the loss of chestnut. They have grown up pretty much without disturbance since. People who drive the parkway up on the ridge above you here look out at the landscape and think that it has always looked like this. It is wonderful that we have this preserved area, but in fact this landscape is probably less than 100 years in the making.

What is oak decline?

The symptoms of oak decline are a progressive dieback from the top down and outside in, on dominant and codominant oaks trees that have proved their competitive metal over the decades. Again, decline is progressive from the standpoint that it may take years or even decades to progress from those initial symptoms to more advanced symptoms. In late stage symptoms you have epicormic sprouts coming off the main stem. There can be a gradation of twig condition, from twigs that still have buds on them, and are very recently dead, to branches that have dieback. But these are signs of a progressive dieback, taking years or even decades, progressing to mortality in susceptible trees. The species in the red oak group are more susceptible to oak decline mortality than those in the white oak group.

According to Sinclair (1965), oak decline etiology begins with factors that predispose the tree to decline (predisposing factors):

- Soil depth and texture
- Species composition
- Competition
- Physiologic age
- Topography
- Climate trends, past events
- Air pollution

These are longstanding conditions that predispose trees to effects that we will discuss. But one in particular, physiologic age, is different from chronological age. An 80-year-old tree is not an 80-year-old tree; it depends on where it is growing. An 80-year-old tree on a poor quality site, or a low productivity site, such a site index of 60, is more mature physiologically than that same age tree growing on a more productive site, say with a site index of 80. And we use this in modeling work to predict where oak decline is likely to be a problem.

The second group of factors are the inciting factors (Sinclair 1965):

- Defoliation
- Drought
- Frost
- Stand disturbance
- Air pollution

These factors are relatively short term, occurring at a point in time or a period of time that can be identified with the inciting event. And defoliation, spring defoliation in particular, is an important factor here. What happens with spring defoliation is that the carbohydrate chemistry of the tree is altered. Food is stored in roots as starch. In times of stress, such as when the crown is removed, the tree has to mobilize that starch into sugars.

Finally, there are the contributing factor (Sinclair 1965), such as:

- Root pathogens
  - Armillaria root disease
- Canker pathogens
  - Hypoxylon
  - Shoot cankers
- Boring insects
  - 2-lined chestnut borer
  - Red oak borer

Root diseases, for example, can take advantage of a tree weakened by inciting factors through recognizing chemical changes in the roots and then switching from a saprophytic to a pathogenic relationship with the tree. These include *Armillaria* root disease, and in particular *Armillaria mellea*..
Using FIA data points of various dissections of forest type, the oak forest type is the most common one in the East, of course, and the message is that "there sure is a lot of oak out there" (Figure 4). When plots are displayed that are 'vulnerable', meaning that they have a relatively high basal area of oak, these are really saw timber and pole timber stands that have a high concentration of oak (using size as a surrogate for age). Vulnerable plots are concentrated in the Appalachian Mountains, the Blue Ridge in Virginia, the Eastern and Western Highland Rims in Tennessee, and the Ozark Mountains in Arkansas. "Affected" stands in Figure 4 are those in which oak decline symptoms are actually present, and these reveal a pattern. There is about 3.6 million acres of oak forest type in the 12 southern states of this region, about 10 percent of the total in the East. Oak and oak decline are especially abundant in the southern Appalachians, where chestnut would have been concentrated.

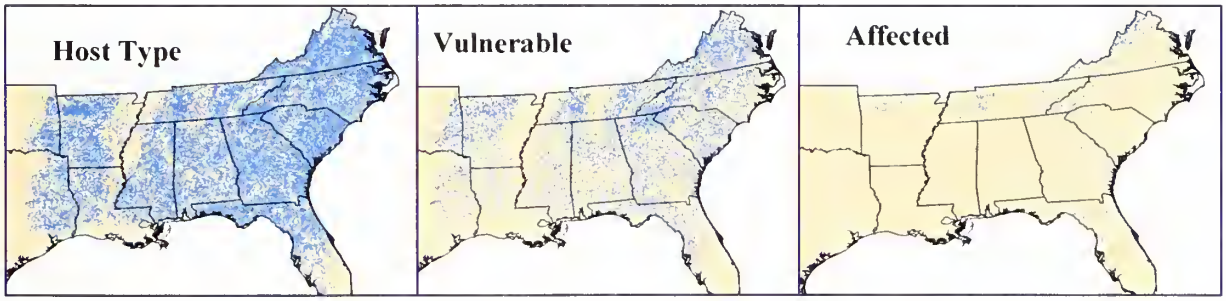

Figure 4. FIA oak decline analysis for the USDA Forest Service's Southern Region, 1984-1989 inventory cycle.

We mentioned earlier that defoliators have an important impact. The fall canker worm is a common defoliator, but probably made more serious by the loss of chestnut and its replacement with oak, as their favorite food is oak leaves. There was an outbreak of fall canker worm on some 10,000 acres on the Blue Ridge Parkway a couple of years ago that resulted in some tree mortality. This is an example of why spring defoliation is important in the oak decline scenario and in general tree health. Oaks produce an instant crown in the spring. If something comes along and removes those leaves in the first few weeks, then the tree has to make a decision about replacing that foliage, and starch needs to be converted to sugars from the roots. Spring defoliation stimulates a refoliation before the starch can be replenished, and

then you get the root diseases coming in. And the lesson also is that compounding stresses such as defoliation in combination with drought unhappily occur together frequently. Nitrogen content in leaves go up in drought periods, which makes it more palatable to insects, a positively-reinforcing loop. When predisposed oaks of advanced age are defoliated in the spring, combined with drought, disaster is waiting.

Another added element is the gypsy moth, a non-native defoliator. The male has feathery antennae and the sex pheromone is from the female, which doesn't fly. Unhappily, the gypsy moth prefers oak species as host; they love to eat oak leaves. Among the more resistant species is the dearly departed American chestnut, and there is another array of hosts that are also relatively immune (Table 2.). Some other immune hosts are species that we do not need necessarily need more of. The bottom line is that the replacement of chestnut, a relatively resistant host to the gypsy moth, with the much more preferred oak again has forest health implications, especially in the oak decline scenario.

Table 2. Tree host preferences for gypsy moth.

Gypsy Moth Preferred Hosts	**Gypsy Moth Resistant Hosts**	**Gypsy Moth Immune Hosts**
Oak species	American chestnut and beech	Ash
Basswood	Cottonwood and sourwood	Fir
Sweetgum	Sweet and yellow birch	Grape and holly
Serviceberry	Hemlock and pines	Black locust
Hornbeam, hop-hornbeam	Blackgum and buckeye	Sycamore
Willow	Walnuts and hickories	Yellow-poplar
Apple	Black cherry and elms	Striped maple
Aspen	Cucumbertree and sassafras	Dogwood
Gray, paper, and river birches	Red and sugar maple	Mountain-laurel

Outbreak frequency, severity, and periodicity tend to be different between native and non-native defoliators, and this has forest health implications. Outbreaks of non-natives tend to be more severe and have shorter return intervals than outbreaks of native species.

So to summarize the chestnut blight-oak decline-gypsy moth interactions, we have an introduced pathogen superimposed on an altered forest due to the loss of chestnut and replacement with oak. That is an oversimplification; oaks weren't the only species to come in, but they were a very significant component to replace chestnuts. But when you impose the interacting factors of oak decline, gypsy moth, forest composition, and existing composition, oaks will decrease as a result of oak decline. This is somewhat site specific. Sometimes oaks replace themselves, but often they don't. The usual case is that there is incomplete oak replacement. So when a forest has 40-60 percent oak prior to these disturbances, you may end up with 20-25 percent remaining afterwards. Nobody projects that oaks will be lost completely; you couldn't get rid of them if you wanted to. There is an increase in the taller, mid-story species, and it is the same scenario as what happened when oaks were positioned to take newly available space when the chestnut went out. You get shade tolerant mid-story species as a result of going decades without disturbance (no fire, no cutting, or very little anyway). You have a build-up in the mid-story of shade tolerant species like red maple, blackgum, and sourwood. Of course, this does not matter if all you want is something green out there. But if you place differential value on different species, then this could be a bad result, especially with regard to wildlife habitat components.

Evidence of forest composition change

Unpublished data from the USDA Forest Service's Forest Inventory and Analysis (FIA) unit was assembled by Bob Anderson, recently retired. This was a study of a cluster of counties in northern Virginia where gypsy moth, oak decline, and dogwood anthracnose have come together over a number of decades. Between 1977-92, approximately three inventory cycles, there was a major change in trees 17 inches and larger in diameter at breast height (Figure 5). The bottom line is that the large-tree component increased dramatically, especially for eastern white pine but other species, also. That is a positive change. But the picture is very different at the other end of the size spectrum. In the trees 1-5 inches in diameter, which are going to be the next forest, over the same period of time, eastern hemlock showed a fairly robust increase but all other species declined. At the bottom, with the most negative changes, were the oak species. So the next forest is probably going to have a smaller oak component.

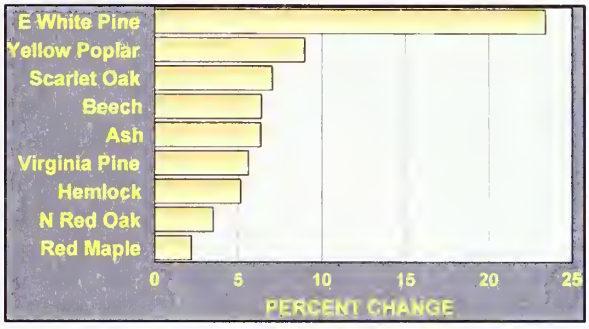

Figure 5. Forest composition in northern VA, change in trees 17+" d.b.h., 1977-92.

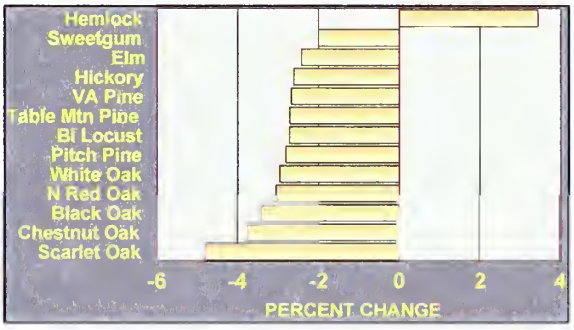

Figure 6. Forest composition in northern VA, change in trees 1.0-4.9" d.b.h., 1977-92.

All of these changes have consequences for wildlife habitat. The mast quality and quantity is reduced, and this has consequences not only for food for mast-loving wildlife, but also in oak regeneration opportunities. This must be put in the context of oak as an incomplete surrogate for chestnut, and what chestnut provided in decades past. We have an increase in small openings, not such a bad thing in some contexts, depending on which wildlife species you are talking about and the landscape you are dealing with. There will be a change in species composition, both in the abundance and diversity of oaks, since the red oak group is more susceptible to the decline mortality than is the white oak group. Reduced canopy density, an increase in denning sites for types of wildlife, and structural changes from dead and downed wood, standing snags and so forth could be a good thing. But how many dead snags do you need in a landscape before they can no longer be exploited by the available wildlife populations? We tried to model what the effect of oak decline would be on acorn production. If all standing trees were alive, healthy, and producing an average amount of mast per year, the annual mast production would be somewhere on the order of 280 pounds per acre. But, of course, many of those trees aren't alive. A real stand was modeled in Virginia, on the Deerfield Ranger District on what is still the GW Jefferson National Forest. Mast production from the dead oak was, of course, zero, and some trees had partial crown dieback and partial reduction in their mast-production capacity. Instead of 280 pounds per acre, the stand was producing 168 pounds on average. Projecting the current pace of decline, knowing that red oaks decline faster than white oaks, we predict that within 10 years of this inventory there will be only 115 pounds per acre. Again, superimpose this on the context of a chestnut forest prior to its loss and replacement with oak. We don't have an accurate number for the mast production of chestnut historically on this kind of a site, but it might have been measured in tons per acre rather than hundreds of pounds per acre.

Sudden oak death and chestnut

How does sudden oak death, or the potential of sudden oak death, fit into this? I tell people that there is a wide spectrum of possible outcomes with sudden oak death, from a chestnut blight type of scenario to innocuous. Sudden oak death was confined to the West Coast (and Europe) until March of this year. It wasn't in the East until the disease (caused by *Phytophthora ramorum*) was shipped on nursery stock to virtually all of the states plus Puerto Rico and the Virgin Islands. However, introduction does not necessarily mean establishment. So what does sudden oak death look like? The diagnostic symptom is a bleeding stem canker on oak, but there are a lot of agents that cause cankers on oak stems. So bleeding cankers are not strictly speaking diagnostic, but a good clue. The bleeding is a running, wine or burgundy colored ooze (Figure 7). Underneath the bleeding spot are irregular lesions. On other species, *P. ramorum* infection may cause only shoot dieback (madrone) or leaf blight (California-laurel). It has been said that sudden oak death is neither sudden, doesn't affect only oaks, and doesn't always result in death. So maybe that is not a good name. But it has crept into the common usage. You would have to say at the low end of the symptom scale that it might be 5 or 10 years from the infection to mortality. We haven't been looking long enough to know if some trees can recover. It doesn't appear so.

Figure 7. *Phytophthora ramorum* diseases – bleeding stem canker, shoot dieback, and leaf blight (clockwise from left).

Prior to March 2004, the distribution of *P. ramorum* in North America was thought to be confined to the West Coast, to 12 central coastal California counties plus Curry County, Oregon, just north of the California border and a couple of hundred miles north of the most northerly known site in California. We tried models to guide our survey efforts, to have a risk-based survey and to focus our resources in places where we were most likely to find this disease. We looked at climatic variables where the disease exists on the West Coast, and combined those with distributions of known potential hosts. As Figure 8 shows, there appears to be a heavy risk of sudden oak death in the southern Appalachians.

On March 10, 2004, it became known or confirmed that *P. ramorum* pathogen was present in the Monrovia nursery in Los Angeles, California. Shipments of nursery stock from Monrovia, and another nursery called Specialty Products, to eastern destinations may have contained infected material. *P. ramorum* has been confirmed in nursery stock sent to Maryland, Virginia, North Carolina, Tennessee, Georgia, Florida, Louisiana, and Texas. Testing is continuing. Just because states are not known to have the shipments yet, does not mean it's not there. It is just that the testing is still underway in many of those places.

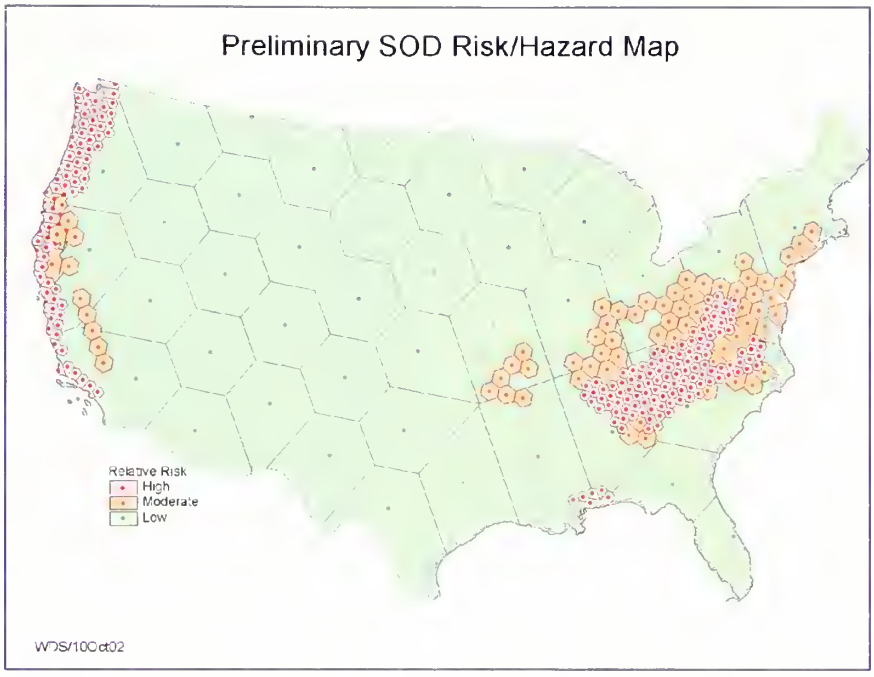

Figure 8. Preliminary sudden oak death risk/hazard map.

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CURRENT STATUS OF CHESTNUT IN EASTERN US FORESTS

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Abstract: The USDA Forest Service, Forest Inventory and Analysis (FIA) program provides the opportunity to assess the current distribution of American chestnut (*Castanea dentata* (Marsh.) Borkh) and prospective trends. Assessing chestnut using the FIA data was challenging because of the coarse nature of the FIA sample and chestnut's rarity in natural forests; however, a basic analysis of location and character provide important information for scientists seeking to re-establish chestnut. Chestnut occurred from Vermont to Alabama or from roughly 45° to 30° north latitude. The estimate of the area of forest land with chestnut at least 1.0-inch in diameter was 2.8 million acres. The area with the highest concentration of chestnut aligned well with Braun's oak-chestnut forest region. About two-thirds of the chestnut sample was on private land and 87 percent was found in oak-hickory stands that vary considerably in composition from north to south. Derivation of a population estimate for the total number of chestnut stems was precluded by missing data. Trends in the existing sample of sapling and tree-size stems suggest a decrease in sapling-size stems and an increase in tree-size stems. Future research on chestnut using FIA data could include filling in data gaps as new inventories are completed, development of improved indicators using new national core health variables, and analysis using geographic information systems (GIS).

Keywords: American chestnut / *Castanea dentata* / distribution / map / Forest Inventory and Analysis / oak-chestnut forest region.

INTRODUCTION

American chestnut (*Castanea dentata* (Marsh.) Borkh.) is still a component of the forest understory in much of its native range despite its extirpation as an overstory component by the chestnut blight (*Endothia parasitica* (Murr.) Anders and Anders) (*Cryphonectria parasitica* (Murrill) Barr.) beginning in the early 1900's (Paillet 1988, Stephenson and Adams 1991). The USDA Forest Service, Forest Inventory and Analysis (FIA) program conducts large-scale forest inventories across the United States and provides the opportunity to assess the current distribution of chestnut and prospective trends. Assessing chestnut using the FIA data was challenging because of the coarse nature of the FIA sample and chestnut's rarity in natural forests. However some basic analyses of location and character can provide important information for scientists seeking to re-establish chestnut. An examination and analysis of available data is provided, along with cautionary comments on data interpretation.

MATERIALS AND METHODS

In 1999, the FIA program converted from a periodic system, in which states were inventoried every 10 to 15 years, to an annual system with fixed portions of a state's forests measured annually. FIA uses a three-phase system to inventory and monitor forests. Phase 1 uses remote sensing to stratify the land base as forest and nonforest and assign a representative number of acres to each sample plot measured in Phase 2.

Phase 2 consists of field measurements collected on a grid of sample plots spread across the United States. Each plot is made up of four 24-foot circular fixed-radius subplots for inventory of trees at least 5.0 inches in diameter. Trees less than 5.0 inches are inventoried on 6.8-foot circular fixed-radius micro-plots nested within each subplot. At each sample plot, a suite of plot and tree-level measurements are collected. Each Phase 2 plot represents about 6,000 acres, although some states have intensified sample grids. Phase 3 measurements are collected on a limited number of Phase 2 locations and include more detailed forest-health parameters, such as tree crown condition.

The Phase 2 sample data were used to identify locations where chestnut occurs and to characterize sites, stands, and tree sizes. Tree-size class provides a surrogate for age or stage of development. Seedlings are trees that are less than 1.0-inch in diameter and at least 0.5 and 1.0 feet in height for coniferous and deciduous species, respectively. Saplings range from 1.0 to 4.9 inches. Tree size is defined as 5.0 inches in diameter and larger. The population estimate of the total forest land acreage with chestnut is mentioned; however, it should be recognized that chestnut's occurrence is rare and discontinuous, so the accuracy and precision of population estimates and related findings are often low.

Other sampling issues associated with chestnut may affect estimates and conclusions. Misidentification can occur because of confusion with Asian chestnuts, cultivars, and similar species. Allegheny chinkapin (*Castanea pumila* (Mill.)) shares much of the current distribution and may have resulted in errors of inclusion. Also, when tallying clumps of seedlings of a single species, FIA crews usually record the most dominant stem. In some older inventories, chestnut may have been grouped into a nonspecific species code. In other cases, seedling tallies were limited to the four most dominant species and only collected if no larger trees occurred on the sample plot. The lack of seedling data for all states was the major limitation of the study. Other less significant factors include differing sample grids, plot designs, and methods of measuring snags among states and inventory dates.

Data were screened for obvious outliers. Less obvious or questionable plots were allowed to remain in the dataset, recognizing that the distribution maps based on these data may contain errors of inclusion or exclusion. Errors of exclusion are often known. For example, FIA field staff in Pennsylvania reported many sightings of chestnut in the vicinity of sample plots, but chestnut was not actually sampled.

Despite these difficulties, FIA data are the only source of consistently gathered sample data on the contemporary occurrence of chestnut throughout its original distribution. Maps of chestnut distribution and related stand characteristics provide useful information for scientists interested in location and extent. More specific local results are available through herbarium studies and other monitoring.

Sources of FIA data used to characterize chestnut came from all available digital data for the states within the natural range of chestnut prior to the blight (Little 1977). This included data from the older periodic inventories and the new annual inventories for states where chestnut appeared in the inventory (Table 1). The most current inventories occurred from 1991 to 2002 and previous inventories from 1980 to 1995. In some cases, only one inventory was available. In order to minimize the amount of error introduced, annual inventory data were used only if at least 50 percent of the sample plots in any given state had been measured. Although this allowed the most current data to be used, some imprecision was apparent in the results. Other source data are contained in the numerous state-level reports published by FIA since the 1930s, but documenting the significant post-blight decline of the early and mid-1900s went beyond the objectives of this study. As such, the analysis covered the current resource and the latest trend information available.

Table 1. Sources of FIA data used to characterize contemporary occurrence and distribution of American chestnut in the eastern United States.

	Previous Inventory				Current Inventory			
State	Year	Inventory type	Number of forested plots	Number of plots with live chestnut[1]	Year	Inventory type	Number of forested plots	Number of plots with live chestnut[1]
Alabama	1990	Periodic	3923	3	2000	Periodic	4421	3
Connecticut	1985	Periodic	215	2	1998	Periodic	319	2
Georgia	1989	Periodic	7713	2	1997	Periodic	7272	4
Illinois	1985	Periodic	1169	0	1998	Periodic	1750	2
Indiana	1998	Periodic	1605	1	1999-2002	Annual	738	0
Kentucky	1988	Periodic	2005	4				
Maine	1995	Periodic	2733	1	1999-2002	Annual	2560	0
Maryland	1986	Periodic	716	3	1999	Annual	562	8
Massachusetts	1985	Periodic	243	1	1998	Periodic	583	14
Michigan	1993	Periodic	10849	0	2000-2002	Annual	4200	1
New Hampshire	1983	Periodic	590	4	1997	Periodic	853	4
New Jersey	1987	Periodic	254	2	1999	Periodic	429	4
New York	1993	Periodic	3063	14				
North Carolina	1984	Periodic	5676	37	1990	Periodic	5965	31
Ohio	1993	Periodic	1802	1				
Pennsylvania	1989	Periodic	3208	53	2000-2002	Annual	1929	19
Rhode Island	1985	Periodic	61	0	1998	Periodic	123	6
South Carolina	1993	Periodic	4563	1	1999-2001	Annual	2815	0
Tennessee	1989	Periodic	2315	4	1999	Periodic	2838	9
Virginia	1992	Periodic	4424	45	1998-2001	Annual	3169	41
West Virginia	1989	Periodic	2628	11	2000	Periodic	2188	21

[1] At least 1.0-inches in diameter.

RESULTS

Current Distribution

Chestnut samples plots were found between about 45° and 30° north latitude, but 85 percent were between 41° and 35° north latitude. Figure 1 depicts sample plots where live or dead chestnut trees at least 1.0 inch diameter were present in any of the inventories since 1980. Plots containing only dead trees were included to provide the most inclusive range description possible. Chestnut occurred from Vermont to Alabama and from Illinois in the west to Maine in the east. It was native to Ontario also, but FIA data do not cover Canada.

The estimate of forest land area with live chestnut at least 1.0 inch in diameter is 2.8 million acres. This estimate is based on the most recent cycle of inventories. The FIA definition of forest land includes areas at least 1 acre in size, at least 10 percent stocked with trees (or has been in the past), in a strip at least 120-feet wide, and not characterized by land uses that inhibit normal forest regeneration and succession (such as mowing). As such, some land with chestnut trees in fencerows or other land with trees would be excluded. It should also be noted that the estimate of forest land with chestnut would be higher if seedlings were included in the analysis.

Figure 1. Distribution of FIA sample plots where live or dead American chestnut trees were found through inventories conducted since 1980. Shaded area represents the Oak-Chestnut Forest Region (Braun 1950).

The top six states by number of sample plots with live trees at least 1.0-inch are Pennsylvania (53), Virginia (45), North Carolina (37), West Virginia (21), Massachusetts (14), and New York (14). Note that this ranking is based on a sample slightly different than the one depicted in Figure 1; this ranking is based on the most recent full periodic inventory of each state to utilize the largest sample possible. As such, larger states have larger samples.

High concentrations of chestnut were found in southern New England (Fig. 2), Pennsylvania, West Virginia-Virginia-Maryland (Fig. 3), and east Tennessee and western North Carolina (Fig. 4). Figures 2 to 4 also show the distribution of samples by tree-size class. It is notable but not surprising that the current extent and abundance suggested by this somewhat fragmented picture aligns with Braun's (1950) oak-chestnut forest region and mixed Mesophytic region to the west.

Site and Stand Characteristics

FIA site and stand data were used to characterize forest stands containing chestnut. The basic findings were similar to those of Braun (1950) who described the extent and abundance following the blight using existing reference sites and standing dead trees.

The contemporary chestnut population occurs across a range of slopes, but was rare on the steepest slopes (Table 2) and most common at elevations below 2000 feet. Chestnut was most prevalent on northeast-facing slopes, but was common on all aspects. About three-fourths of the chestnut occurred on mesic sites. It was also found on xeric sites. Chestnut was rare on very wet sites.

Forest resources in the eastern United States are primarily controlled by private forest landowners and stands containing chestnut are not an exception. Nearly 60 percent of the chestnut sample was on private land. The private owner group is a complex mix, from timber industry to small family owners. The National Forest System was the second most common owner with 25 percent of the total chestnut sample. The other public sample is incomplete because the existing sample excludes National Park Service land in the North Carolina portion of the Great Smoky Mountain Park and the Adirondack and Catskills State Parks in New York. New inventories will cover these lands in the future.

As was similarly found by Braun (1950), chestnut occurred most commonly in the oak-hickory forest-type group. The oak-hickory group covers a wide range of forest cover types, primarily of mixed-oak composition with varying proportions of other associates, such as yellow-poplar (*Liriodendron tulipifera* L.), hickory (*Carya* sp.), and other species depending on the region. More than 80 percent of the sample plots with chestnut were found in oak-dominated stands. Although found across the East, high concentrations of oak-dominated stands are very common in the Appalachian Mountains from central Pennsylvania to northern Alabama (McWilliams and others 2002).

In southern New England, chestnut occurs on glaciated soils with higher occurrence of red maple (*Acer rubrum* L.), sugar maple (*A. saccharum* Marsh.), beech (*Fagus grandifolia* Ehrh.), and white ash (*Fraxinus americana* L.) than regions to the south. The northern Ridge and Valley region of Pennsylvania is characterized by mixed-oak species with yellow-poplar and hickory being relatively rare. Yellow-poplar and hickory become more common in stands containing chestnut in the southern tier of Pennsylvania and the northern Blue Ridge areas of West Virginia and Virginia. Further south in the Southern Appalachians and Great Smoky Mountains, the number of associates increases. These differences in associates emphasize the high degree of species heterogeneity that exists throughout eastern North America.

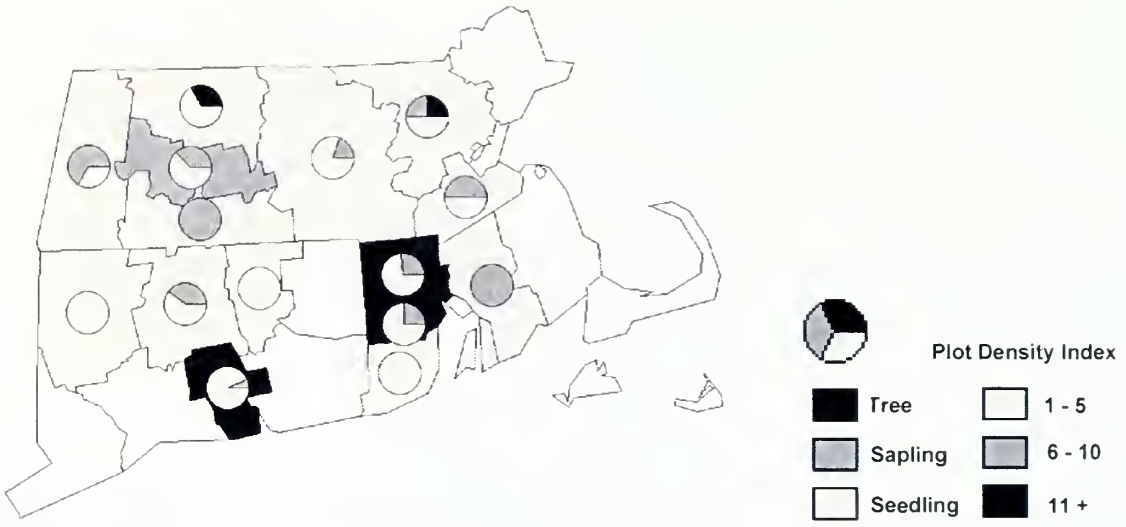

Figure 2. Distribution of live American chestnut showing chestnut plot density index and proportion of seedlings, saplings, and trees by county in Southern New England. [Note: Plot density index = number of plots with live chestnut/county area (sq. mi.) * 1000].

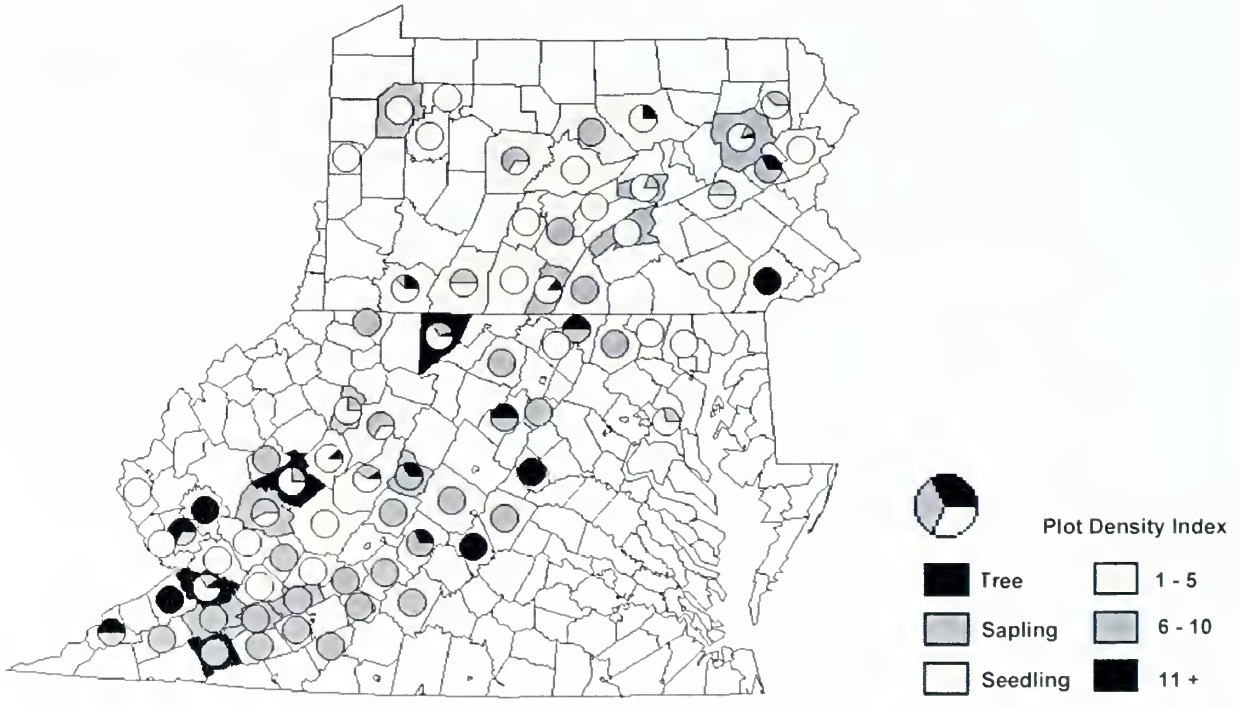

Figure 3. Distribution of live American chestnut showing chestnut plot density index and proportion of seedlings, saplings, and trees by county in Pennsylvania, Maryland, West Virginia, and Virginia. No seedling data available for Virginia. [Note: Plot density index = number of plots with live chestnut/county area (sq. mi.) * 1000].

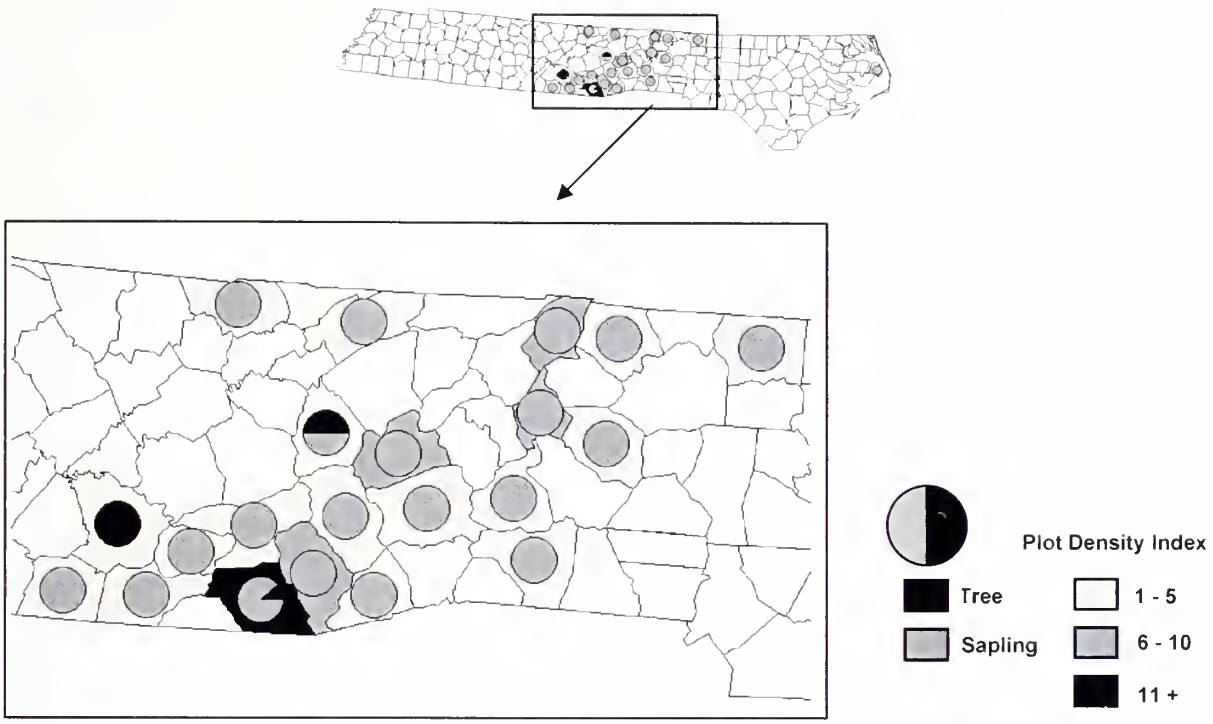

Figure 4. Distribution of live American chestnut showing chestnut plot density index and proportion of saplings and trees by county in east Tennessee and western North Carolina.
[Note: Plot density index = number of plots with live chestnut/county area (sq. mi.) * 1000].

The distribution of forest land containing chestnut by stand-size and stocking class was similar to the distribution for all forest land across its range. By stand-size class, the distribution was 60 percent sawtimber size, 28 percent mid-size, and 12 percent sapling-seedling size. Sawtimber stands are dominated by trees at least 9.0 and 11.0 inches in diameter for coniferous and deciduous species, respectively. Mid-size stands are dominated by trees at least 5.0-inches in diameter but smaller than sawtimber size. Sapling-seedling stands contain mostly trees less than 5.0 inches. Eighty-eight percent of the forest land with chestnut was in either medium (30-69 percent stocked) or fully (70-100 percent) stocked stands.

Large areas of eastern U.S. mountain and upland forests are evolving along similar compositional and structural trajectories. Stands containing chestnut are representative of these conditions. Dominant trends include forest land with increasing numbers of large-diameter trees, decreases in small to mid-range trees, mismatches between overstory and understory species composition, relatively few young sapling-seedling stands, and often, regeneration difficulties. Susceptibility to prominent pests, such as Asian long-horned beetle, elm-ash borer, and hemlock wooly adelgid threaten many of the canopy dominants that occur over significant areas. Future developments in these forests will affect chestnut's niche within natural and disturbed forest land.

Table 2. Site and stand characteristics expressed as a percent of plots with live American chestnut (at least 1.0-inches in diameter) sampled in the most recent FIA inventories conducted in the eastern United States.

--- Percent of Sample Plots ---

Slope		Elevation		Aspect	
0-5%	12	500'	8	NE	33
6-10%	10	1000'	38	SE	20
11-20%	19	1500'	17	SW	22
21-30%	14	2000'	14	NW	24
31-40%	15	2500'	11		
41-50%	14	3000'	6	Moisture Class	
51-60%	10	3500'	6		
61-70%	4	4000'	1	Hydric	- trace -
71-80%	2			Mesic	73
				Xeric	27

Ownership		Stand Size	
National Forest	25	Sapling-Seedling	12
Other Federal[1]	2	Mid-size	28
Other Public	14	Sawtimber	60
Private	59		

Forest Type Grouping		Relative Stocking	
White Pine-Hemlock	2	Over (>100%)	8
Spruce-Fir	-trace-	Full (70-100%)	55
Loblolly-Shortleaf	2	Medium (30-69%)	33
Oak-Pine	5	Low (< 30%)	3
Oak-Hickory	84		
Elm-Ash-Cottonwood	1		
Northern Hardwoods	6		
Aspen-Birch	1		

[1] The Other Federal ownership excludes forest land with chestnut in the Great Smoky Mountain National Park and the Adirondack and Catskills State Parks.

Numbers of Stems

Derivation of a population estimate for the total number of chestnut stems was precluded by some missing information for seedlings. However, an examination of the existing sample of sapling and tree-size stems suggests a decrease in sapling-size stems and an increase in tree-size stems over the two most recent inventory cycles. It is unfortunate that the seedling sample was incomplete because structural trends are not completely discernable. The critical question is what degree the seedling/sprout resource is changing. This resource represents recruitment of future chestnut stems. While the increase in tree-size stems is encouraging in terms of viability, the long-term sustainability of chestnut depends on recruitment of chestnut stems.

CONCLUSIONS

The FIA data provide a coarse description of the chestnut resource as it occurs in today's forests. The data indicate that the existing population of chestnut occupies the core of the oak-chestnut forest region described by Braun (1950) with relic communities found across its original range described by Little (1977). This is not surprising because chestnuts exist today mainly as sprouts (Paillet 1988). It is not possible to make a conclusive statement of the long-term sustainability of chestnut due to limitations of the current dataset. Future inventories will fill existing gaps and provide additional data needed for more thorough analysis of structural changes and trends in spatial extent. A significant benefit of the new national FIA system is improvement to the seedling and sapling measurement protocols. All seedlings are now tallied in a consistent manner. Sapling measurements now include total height, crown class, and condition. Remeasurement of these parameters will lead to improved datasets over the next 5 to 10 years.

Future extensions of research on chestnut using FIA data are readily apparent. The most obvious need is to provide more comprehensive data for analysis. Pending release of FIA results for Kentucky, North Carolina, and New York will fill some critical needs. The inclusion of seedling information in FIA's current national protocols will be particularly helpful. Once the gaps in data are filled, a more complete analysis of site occupancy could be conducted using geographic information systems (GIS). Modern GIS software is capable of analyzing hundreds of data layers that could help delineate characteristics associated with chestnut's occurrence. Improvements to FIA Phase 2 and new Phase 3 variables have resulted from nationalization of FIA protocols. For example, Phase 3 includes tree crowns, damage, down woody material, and others. These new variables offer the opportunity to develop improved indicators of chestnut condition and extent. The opportunities for improvement of our knowledge of chestnut at the landscape level are immense.

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CHESTNUT AND WILDLIFE

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Abstract: The interaction of chestnut with wildlife can be separated into two issues: 1) Chestnuts as a resource for wildlife consumption; and 2) Wildlife as a dispersal agent for chestnut seed. The chestnut mast resource was probably not qualitatively different from that of other large-fruited mast species. Chestnut mast may have been quantitatively better because chestnut crops were probably more reliable from season to season, and because chestnut has been partly replaced by tree species such as tulip poplar that do not produce nuts. These factors may result in a 20 to 50% increase in total mast production if chestnut is re-established as a canopy-dominant tree in American forests. Dispersal of chestnut seed and seed burial by wildlife were important because chestnuts have little resistance to frost or desiccation. However, chestnuts as seed exhibit few of the mechanisms usually associated with a trade-off between dispersal and predation. Various field studies and old forestry data confirm that chestnut reproduction occurred most often by coppice sprouting, and that chestnut seedlings were rare or absent in many chestnut stands. I propose that there is no selective pressure on chestnut for the deterrence of predators because chestnut reproduction is based on a combination of wildlife dispersal of nuts and long term survival of the few seeds that do manage to germinate. My studies show that many chestnut seedlings have survived for at least a century and possess root collar sprouting characteristics designed to insure that "old seedlings" remain juvenile essentially forever. Thus, chestnut reproduction by seed is heavily biased in favor of mechanisms to promote long-distance transport. The near immortality of established chestnut root stocks probably offsets any selective value in deterring seed predation within chestnut-dominated stands.

INTRODUCTION

American chestnut, *Castanea dentata*, was once one of the leading tree species in the forests of the Appalachians, Allegheny Plateau, and southern New England (Smith, 2000; figure 1). Mature chestnut trees were removed from these forest ecosystems after the introduction of chestnut blight in the New York City area sometime around 1900 (Anderson, 1974). Chestnut mast was completely removed from eastern forests, although numerous chestnut sprouts continue to exist and even flourish in the forest understory (Paillet, 2003). A related species, Allegheny chinquapin (*Castanea pumila*), was also affected by blight, but chinquapin grows as a shrub or small tree, so that some fruiting probably continued to occur even after the appearance of blight within the range of that species (Paillet, 1993). I therefore conclude that blight effectively eliminated seed reproduction by chestnut, and drastically limited seed reproduction on the part of chinquapin.

The elimination of a significant chestnut mast crop from American forests had two important effects: loss of an important food source for wildlife, and loss of the mechanism for seed dispersal on the part of the two native Castanea species. This report addresses the relationship between wildlife and chestnut by considering these distinct issues. The analysis begins by assessing what is known about chestnut ecology and the character of the chestnut mast crop in the years before blight arrived in America. These facts are the used to project how a restoration of that resource might affect wildlife in future forests. The projection is made possible by the general similarity of chestnuts to large acorns in terms of size, seed packaging, and nutrition (grams of carbohydrate, fat, and protein per kilogram, Krockmal and Krochmal, 1982). The analysis then considers how wildlife predation on chestnut seed might affect chestnut

dissemination and reproduction. This, in turn, requires consideration of both the physiology of chestnut production and the ecological factors related to chestnut seedling establishment in the wild.

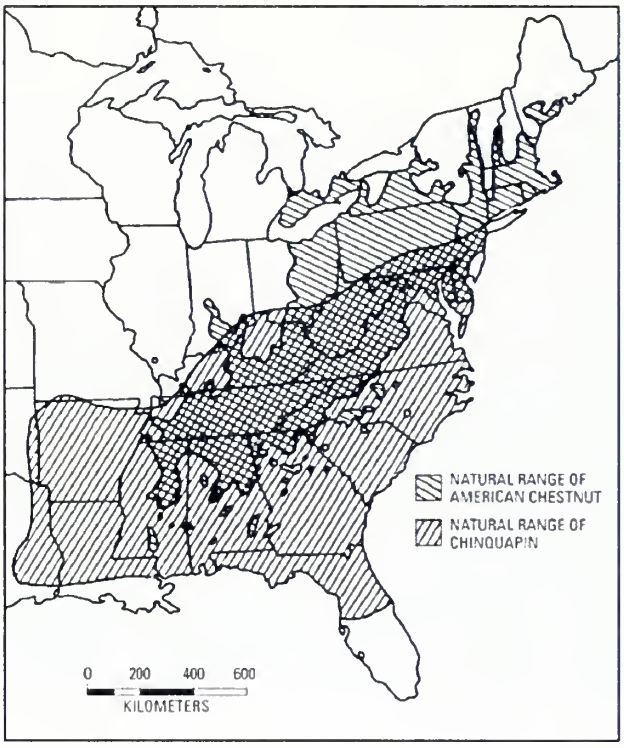

Figure 1. The natural range of chestnut and chinquapin in North America (from Paillet. 2003)

THE FACTS WE HAVE TO WORK WITH

Although there are no natural American chestnut forests to study using the techniques of modern ecology, several different avenues of investigation provide some information about the role of chestnut in pre-blight American forests. These methods include: 1) Analysis of early forestry literature; 2) Pollen analysis from bogs and ponds that were once surrounded by chestnut forests; 3) Study of naturalized stands of American chestnut established beyond the range of blight and natural forests of related chestnut species in Russia and China; and 4) Reconstruction of conditions in former chestnut forests by examination of stumps and surviving sprouts. The known facts can be summarized as follows:

Chestnut was a leading species in eastern North America, often approaching 50% of total stand basal area in upland forests from North Carolina to Connecticut (Nichols, 1913; Frothingham, 1912; Zon, 1904; Buttrick and Holmes, 1913). Chestnut was successfully propagating in natural forests both before and after the arrival of Europeans.

Pollen studies demonstrate that chestnut was one of the last deciduous tree species to arrive within its natural range in the Holocene from a glacial refuge probably located on the southern coastal plain (Davis, 1969; Whitehead, 1979). The best estimate of the glacial habitat of chestnut includes the well-drained bluffs along rivers draining western Florida and Georgia (Watts, 1979).

Chestnut was only found growing on non calcareous soils, and was rare on soils developed on clay-rich glacial till (Russell, 1987). The survival of isolated chestnut trees on till soils in the Midwest demonstrates that the aversion to heavy soils is probably not the result of toxicity, but is caused by a combination of inability to compete and microsite conditions (Paillet and Rutter, 1989)

Pollen studies show an abrupt expansion of chestnut at about 3000 years ago in New England (Brugham, 1978; Whitehead, 1979; Foster and Zybryk, 1991). Pollen ratios shift from less than a few percent to 20% or more. Chestnut pollen is under-represented in pollen profiles by a factor of 3 (Paillet et al, 1991) and yet relatively small chestnut pollen particles can be transported long distances. Thus it is unclear whether chestnut was present in low numbers in New England and upstate New York before 3000 years ago, or the small amount of chestnut pollen present before then resulted from long-distance transport. The 3000 year old shift in chestnut pollen corresponds almost exactly with an increase in spruce pollen in the same catchments, suggesting a climate change may be involved (Davis et al, 1980).

The introduction of European land use practices apparently affected chestnut because many pollen profiles show a near doubling of the proportion of chestnut coinciding with the arrival of European settlers (Brugham, 1980). This is almost certainly a result of the conversion of bottomland soils to fields or pasture, and the changes in disturbance regime on remaining upland woodlots.

Chestnut was destroyed as a forest tree by blight over the period 1900-1950 throughout its natural range (Anderson, 1974). All evidence suggests that the chestnut seed crop was completely removed from the forest as a source of food for wildlife and as a source of seed propagation for the forest (Paillet, 2003).

Even though chestnut trees were destroyed, chestnut sprouts are abundant in modern forests. In fact, chestnut sprouts are so pervasive that they consistently show up a significant contribution to shrub cover (Adams and Stephenson, 1983; Boring et al, 1981).

Chestnut sprouts grow as fast as or faster than the sprouts from other competing species when released by disturbance (Adams and Stephenson, 1983; Stephens and Waggoner, 1980). Chestnut sprouts are recognized as the leading component of biomass in the years immediately after clear cutting in some Appalachian forests (Boring et al, 1981). This ability represents a strong adaptive advantage in "sprout hardwood" forests where canopy regeneration is dominated by coppice sprouts (Hibbs, 1983)

Early historic forestry practices were based on coppice sprout regeneration (Buttrick and Holmes, 1913; Matoon, 1909; Smith, 2000). Older references clearly and repeatedly indicate that chestnut propagation from seed was not effective and that woodlots should be managed so as to encourage regeneration of chestnuts by stump sprouting.

Chestnut has a significant range overlap with chinquapin. Both species reproduce by sprouting as well as by seed. The one major difference is that chestnut (a large forest tree) sprouts only from pre-formed buds on the root collar, whereas chinquapin (a large shrub or subcanopy tree) sprouts from an extend region of the lower stem and upper root system (Paillet, 1993).

THE CURIOUS CASE OF THE ANCIENT SEEDLINGS

Although much of the early forest literature and folklore refers to "chestnut trees smoldering at the roots", careful study of surviving chestnut spouts shows that almost all of these sprouts are old seedlings. The old forestry literature indicates that chestnut sprouts only from the root collar and not from roots at a distance from the stump as in the case of aspen and beech (Matoon, 1909; Zon, 1904). Chestnut wood is resistant to decay so that the remains of blight-killed trees can be recognized in the field (Saucier, 1973).

Thus, it is possible to determine whether surviving chestnut sprouts originated from former trees. Such analysis shows that almost all living sprouts never were attached to a canopy-dominant tree (Paillet, 1984). Most sprouts survive for an extended period as small upright trees 2-4 m in height, with an enlarged root collar covered with suppressed buds (figure 2). Maps of dense sprout population show that only a small number originated from the base of former trees (figure 3).

These results indicate that the many living chestnut sprouts in modern forests are old seedlings that have survived for many years without ever becoming a large tree. The remains of large chestnut trees on the site illustrated in figure 3 were killed in 1922 according to the cross-correlation of ring widths with standard chronologies (Paillet, 1984). In addition to simply surviving, the many chestnut sprouts retained their small tree form through several cycles of stem destruction by blight or mechanical damage. Paillet (1993) suggests that this is not coincidence, and indicates this is an adaptation to insure that established chestnut seedlings remain "perpetually juvenile" (figure 4). This cycle of stem regeneration and root system abandonment appears designed to maintain seedling form indefinitely as a method of advanced regeneration. As a result of this sprouting mechanism, an established chestnut seedling might be able to assume its place in the canopy a century or more after germinating from a chestnut deposited in the forest litter. Although other American trees species are capable of producing coppice sprouts, the controlled release of stems and systematic replacement of the roots system in the chestnut seedling sprout cycle is unique to *Castanea dentata*.

CHESTNUT AS A RESOURCE FOR WILDLIFE

Chestnuts must have been a nearly ideal food for mast-consuming wildlife. Ripe chestnuts are not protected by a predator-resistant husk or shell, or by chemicals such as tannins. The general food "package" presented by chestnut was probably similar to that of the northern red oak or swamp chestnut oak (oak species producing relatively large fruit) in terms of fruit size and quantity of seed produced by an individual tree. The nutritional value of chestnuts (grams of carbohydrates, proteins, and fat per kilogram of nuts; Krochmal and Krochmal, 1982) is comparable to that of various oak species. Chestnut canopy crowns in naturalized stands of American chestnut are qualitatively similar to large oaks, and probably produce about the same number of nuts per branch tip as a northern red oak when the tree is producing at full capacity. These arguments suggest that a chestnut-dominated woodlot produced a nut crop that was qualitatively similar to the crop produced by an analogous stand of northern red oak trees.

Restoration of chestnut to American forests might still have an effect on wildlife by making a quantitative difference in total mast crop. An increase in total mast could be produced by two mechanisms: 1) chestnut seed crops would be more regular than those of other nut producing trees; and 2) chestnut might displace some tree species that do not produce nuts. Chestnut seed crops would exhibit relatively few years of low production because chestnut flowers in early summer when there is no possibility of frost damage to ovaries and catkins, and because the nuts mature quickly so as to minimize exposure to insects or other damage to the immature fruit. This would produce a net increase in mast crop when averaged over several years, and could have a beneficial effect for wildlife in those years when a late frost diminishes other nuts. Chestnut is adapted for relative well-drained sites, and would displace mostly other nut producing trees like oak and hickory on the drier end of the spectrum. However, chestnut can also grow on more mesic sites where it mixes with beech, tulip poplar, maple, basswood, and hemlock. Of these trees, only beech produces a significant mast crop. Together, these mechanisms suggest that reintroduction of chestnut might increase total nut production for wildlife by something like 20 to 50%.

Figure 2. Typical chestnut sprout living in the understory of former oak-chestnut woodland in New England; the log leaning against the stone wall represents the typical appearance in 1983 of a chestnut tree killed by blight in 1922.

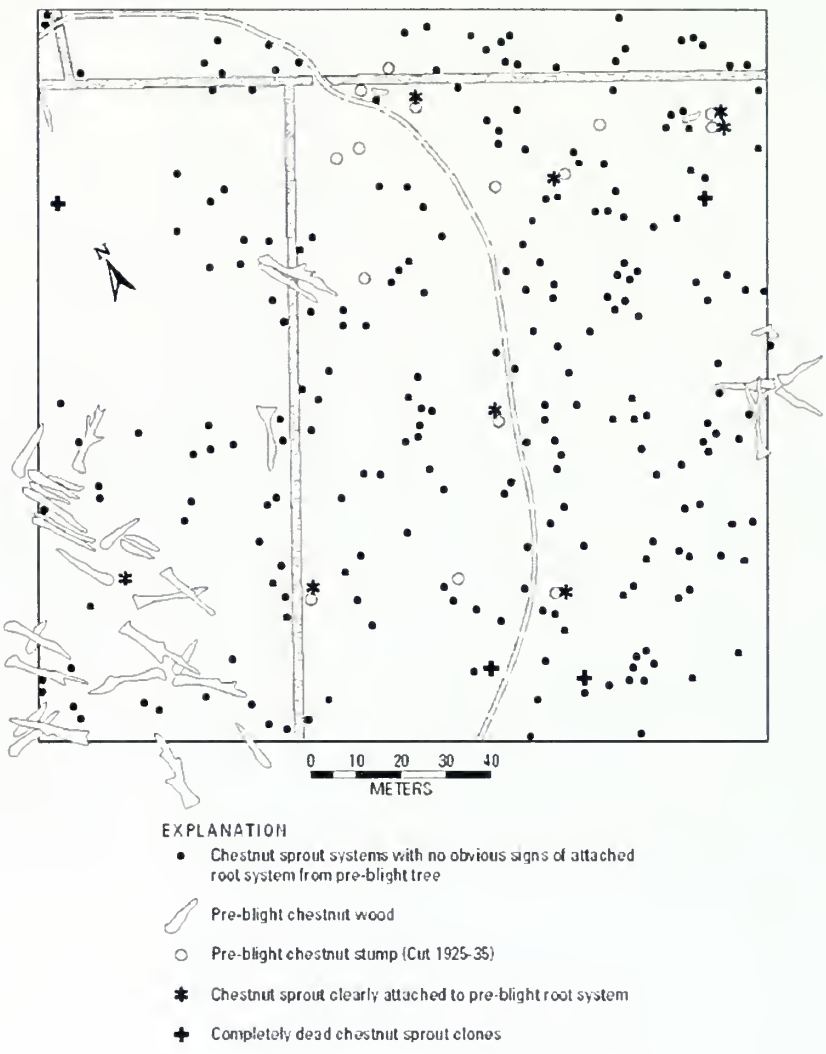

Figure 3. Remains of chestnut trees killed by blight in 1922 and living chestnut sprouts present in 1983 on a one-hectare plot in Andover, Massachusetts (from Paillet, 1984)

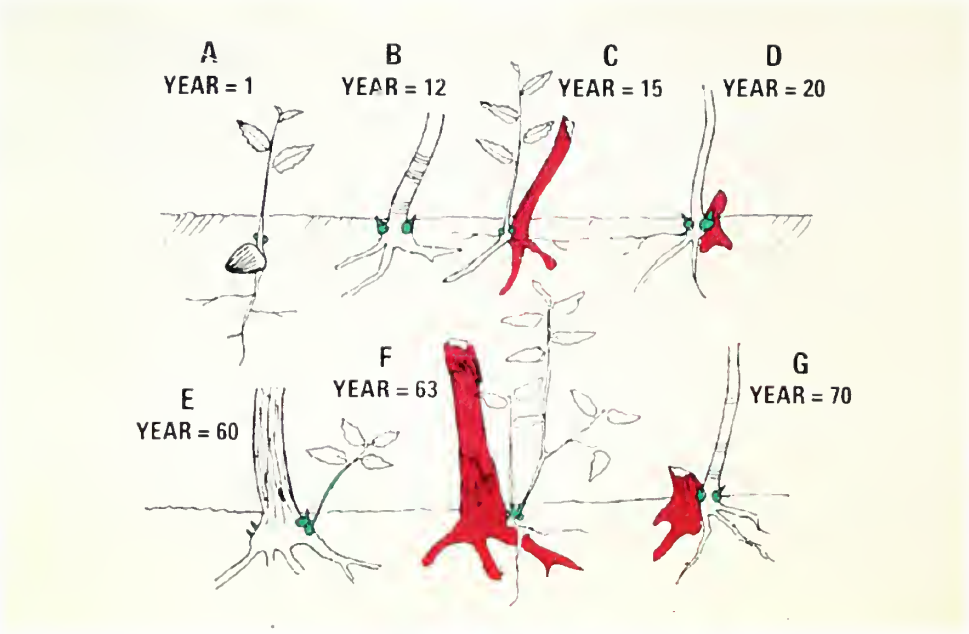

Figure 4. Schematic illustration of the chestnut seedling growth cycle where seedlings develop root collar buds that replace injured or senescent stems, generate a new root system and root collar buds, and retain juvenile growth form and defect-free stem base throughout an indefinite number of such stem replacement cycles.

WILDLIFE AS A DISPERSAL AGENT FOR CHESTNUT

Ecological literature assumes that mast crops are "designed" to be attractive to wildlife to provide effective dispersal of seed. Seed predators and trees interact in complex ways to insure that predators are "rewarded" for their role in planting nuts, while seed protection mechanisms insure that at least some nuts survive to germinate and grow. The seasonality of nut crops naturally results in seed caching by some predators to make the mast crop last the entire year. But this also allows other predators to develop rooting behavior to "harvest" these caches. Thus it is beneficial for nut-producing trees to develop features that deter seed predators. The most often cited mechanism is the irregularity of seed crops from year to year. Most nut-producing trees (oak, hickory, walnut, beech) flower in the early spring. This allows wind to transport pollen through the bare canopy, and causes late frost and spring storms to damage seed crops in certain years. Ecologists suggest that the natural cycling of the seed crop serves to keep predator populations under control. Nuts are also protected by thick, hard seed coats and/or chemicals such as tannins.

Chestnut is unique among nut producing trees in North America in having essentially no deterrence for seed predators. The seed itself is protected by a thin shell and contains no tannins or other chemicals to reduce palatability. Young chestnuts are protected by a formidable burr during development, but the burr opens wide when the seed is ripe. The maturing fruit is exposed to possible damage for a relatively short period and is protected from predation by its burr during that period. Chestnut flowers in late June or July when there is no possibility of frost. All of these factors show that chestnut fruit is produced with regular seed crops that are designed to be as attractive to predators as possible. This presents a paradox in that chestnut seems to defy the logic of predator/prey cycles.

Various studies suggest that seed predation is a real problem in chestnut reproduction. All of the early forestry literature cites a lack of chestnut reproduction by seed. Buttrick and Holmes (1913) suggest that lack of seedling establishment may be the cause of the loss of chestnut from some North Carolina piedmont sites where it was formerly abundant. Thoreau (1906) likewise describes a nearly complete lack of chestnut seedlings in chestnut woodlots in Massachusetts. Paillet (1988) noted that there were some New England sites where logs and stumps showed that chestnut was once dominant in the canopy, but where the low density or lack of living sprouts shows that chestnut seedlings were not being established. He also noted that maps of living chestnut sprouts indicated microsites such as fences, rights of way and brush thickets influenced seedling establishment. Pridnya et al (1996) note a similar condition in old-growth European chestnut forests in southern Russia. Since it is unlikely that these forests were not producing chestnuts and since there is no obvious reason why some seedlings would not germinate, seed predation by livestock or wildlife is assumed to have caused the lack of surviving seedlings. Such observations leave no doubt that seed predation can be a significant problem for chestnut reproduction. The nearly complete lack of any deterrence with regard to seed predation and the apparent effects on seed predation for at least the short term in many historic woodlands presents an ecological paradox that may have implications in attempts to restore naturally-reproducing chestnut trees to American forest.

A POSSIBLE EXPLANATION

My hypothesis addresses this paradox under the assumption that chestnut is a successful forest tree and recognizable as a distinct genus in the fossil record for at least 50 million years (Graham, 1990). If chestnut is a successful species, why has competitive interaction with other forest trees not selected seed traits for factors that deter seed predation? The simplest answer is that such adaptations have not arisen because there is no significant adaptive advantage to them. I suggest that the unique seedling re-sprouting capability of chestnut produces "immortal" seedlings that can survive for centuries. I propose that the longevity of viable seedlings (viable in the sense that they can be released to form a cleanly-formed, canopy-dominant stem) completely compensates for seed predation. At the same time, the extended life cycle of chestnut as a tree (centuries in the understory and then several centuries in the canopy) implies that chestnut "invasion" of new territory is a slow process. This, in turn, places added significance on mechanisms for seed dispersal. I conclude that seed predation is not important for chestnut because the longevity of a few established seedlings compensates for predation. At the same time, the inherent slowness in forest turnover associated with this process places a premium on seed dispersal. Chestnut is "designed" to encourage seed transport in every way, and is largely unaffected by seed predation in the long term.

This explanation is largely a "default option" that suggests chestnut does not deter seed predation because such deterrence has a metabolic cost but has no selective advantage. Is there any positive support for this idea? One line of evidence comes from the comparison of chestnut pollen on two adjacent sites in Massachusetts (Foster and Zybruk, 1991). The data give profiles for a bog catchment receiving pollen for many square km around the site, and a forest hollow catchment underneath the forest canopy receiving mostly local pollen from directly above. The bog pollen shows a rather consistent regional proportion of 10% chestnut pollen over the past 3000 years. In contrast, the forest hollow pollen shows fluctuations from near zero to 60% or more that follow discrete disturbance events (fire and windstorm). This suggests that the regional proportion of chestnut was constant, but that the local abundance of chestnut was highly variable. I interpret this as a situation where seed predation concentrated on the areas immediate around and beneath mature trees, while chestnut seedlings could escape predation in other parts of the forest. This is exactly the same situation as reported by Thoreau (1906), where he could not find chestnut seedlings in chestnut woodlots, but found them abundant in adjacent old-field pine stands. Russian ecologists identify a similar "bottleneck" in chestnut reproduction related to the need to establish chestnut seedlings in the understory (Pridnya et al, 1996; figure 5)

Does any of this have a bearing on the introduction of blight resistant chestnut into national park forests? This hypothesis explains why chestnut is such a slowly migrating species under the influence of climate change. Introduction of blight-resistant chestnut would also be a lengthy process. Several approaches could be used to hasten the process. First, one could use special seed-predator exclusion techniques to produce microsites suitable for chestnut seedling establishment. One could also transplant seedlings to generate established old seedlings to bypass the reproductive "bottleneck" related to seedling establishment. Then one could also rely on the artificial generation of disturbance to promote the release of suppressed seedlings. Although such manipulation may seem ill suited for wilderness areas in national parks, the alternative is a period of 1000 years or more before restored chestnut reaches a natural equilibrium with the surrounding forest.

SUMMARY

The interaction of chestnut with wildlife can be separated into two issues: 1) Chestnuts as a resource for wildlife consumption; and 2) Wildlife as a dispersal agent for chestnut seed. Making an analogy between the growth form and nut crop of northern red oak and chestnut, the former chestnut mast resource is estimated to be qualitatively similar to that of other large-fruited mast species. Chestnut mast may have been quantitatively better because chestnut crops were probably more reliable from season to season, and because chestnut has been partly replaced by tree species such as tulip poplar that do not produce nuts. These factors may result in a 20 to 50% increase in total mast production if chestnut is re-established as a canopy-dominant tree in American forests. The availability of chestnuts in years of frost or insect damage to acorns may make chestnut an especially valuable addition to the forest. Dispersal of chestnut seed and seed burial by wildlife was important because chestnuts are relatively fragile and have little resistance to frost or desiccation. However, chestnuts as seed exhibit none of the mechanisms usually associated with a trade-off between dispersal and predation. Chestnut reproduction occurred most often by coppice sprouting, and chestnut seedlings were reportedly rare or absent in many chestnut stands. One explanation for this apparent paradox is that there is no selective pressure on chestnut for the deterrence of predators. This happens because chestnut reproduction is based on a combination of wildlife dispersal of nuts and long-term survival of the few seedlings that do manage to germinate. Many living chestnut seedlings have survived for at least a century as small suppressed stems and possess root collar sprouting characteristics designed to insure that "old seedlings" remain juvenile essentially forever. Thus, chestnut reproduction by seed is heavily biased in favor of mechanisms to promote long-distance transport. The near immortality of established chestnut rootstocks could offset any selective value in deterring seed predation within chestnut-dominated stands.

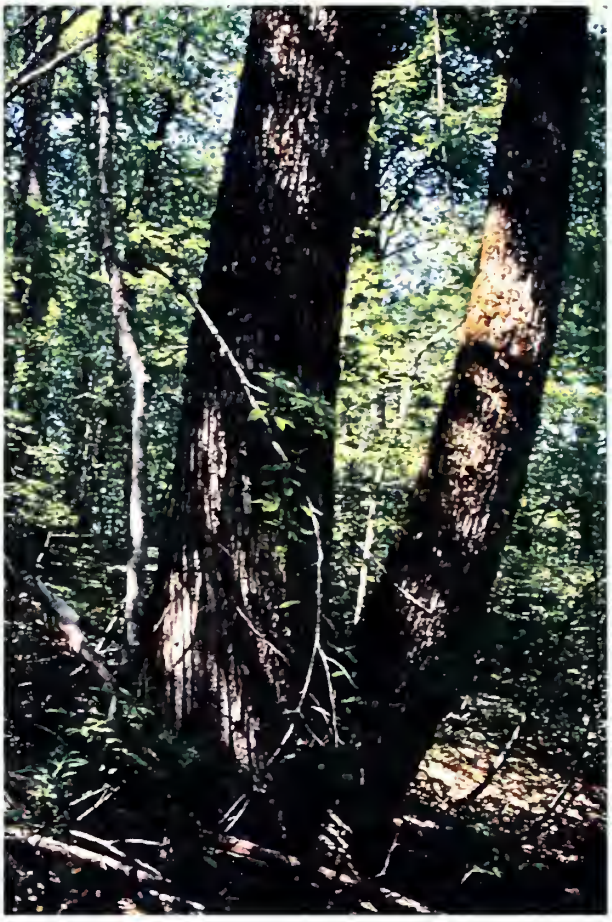

Figure 5. Many European chestnut (Castanea sativa) trees in old growth forests of southern Russia show multiple stems indicative of origin as sprouts derived from suppressed seedlings (from Pridnya et al, 1996).

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HISTORICAL SIGNIFICANCE OF AMERICAN CHESTNUT TO APPALACHIAN CULTURE AND ECOLOGY

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Abstraet: This paper explores the signifieanee of the Ameriean ehestnut on the eeology and eulture of Appalaehia. Until the third deeade of the 20th century, the tree was the erowning glory of the Appalaehian hardwood forest, in some isolated areas eomprising half of the hardwood tree population. The wildlife of the region, particularly blaek bears, heavily depended on the tree for both sustenance and shelter. Native Amerieans in the mountains frequently made use of the nut, mixing ehestnut meal with eorn to make bread. White mountaineers gathered the nuts to sell or trade, and sometimes used pareheb ehestnuts as a eoffee substitute. The American ehestnut played a major role in the eeonomy of the Appalaehian region, providing timber for dwellings and tannie aeid for the leather industry. Finally, it is argued that the decline of Appalaehian subsistenee eulture is direetly linked to the loss of the Ameriean ehestnut.

INTRODUCTION

Few single events in North Ameriean environmental history eompare with the loss of the Ameriean ehestnut. "The devastation of the Ameriean ehestnut by the ehestnut blight," wrote William MaeDonald more than two deeades ago, "represents one of the greatest reeorded ehanges in natural plant population eaused by an introdueed organism." MaeDonald, a professor of plant pathology at West Virginia University and the eurrent aeting treasurer of the American Chestnut Foundation, estimates that ehestnut-dominated forests onee eovered 200 million acres of land from Maine to Mississippi (MaeDonald 1978; Brown and Davis 1995). Ameriean ehestnut trees onee eomprised roughly twenty pereent of the Appalaehian forest, although in speeifie areas they aeeounted for as mueh as one-third of all trees (Kulman 1978). In faet, William Ashe reported seeing several locales in western North Carolina where the trees "oeeur pure or nearly pure over areas as large as 100 acres" (Ashe 1912). In 1901, he and Horaee Ayers estimated that the southern Appalaehians eontained more than 884,000 aeres of ehestnut timber (Ayres and Ashe 1905).

The Ameriean ehestnut was eonfined largely to the Blue Ridge Mountains and Cumberland Plateau where the trees commonly grew at altitudes between 1,000 and 4,000 feet. Aeeording to Charlotte Pyle, the American ehestnut eovered no less than 31 percent, or 159,165 aeres, of what is today the Great Smoky Mountains National Park (Pyle 1985; Davis 2000). The Ridge and Valley physiographie provinee had a few important stands of chestnuts as well, but these were found only on the slopes of the highest ridges where richer soils and heavier rainfall predominated. A notable exeeption was a nearly one-square mile area of Walker County, Georgia, still known today as Chestnut Flats. Aeeording to historian James Sartain, the area "was so-ealled beeause of the abundance of ehestnuts that grew in that beautiful valley and on the adjaeent ridge when the early settlers arrived" (Sartain 1972). A reeonstruetion of nineteenth eentury forests in other parts of northwest Georgia, however, found ehestnut trees eomprising no more than six pereent of the area, with oaks and hiekories, the most dominant tree speeies, making up 45 pereent of the total forest (Plummer 1975).

As is now well-documented, the death of the American Chestnut was due to an exotic blight introduced in the United States from Japanese Chestnut nursery stock just after the turn of the century. A forester at the New York Zoological Park first reported the disease in 1904, after observing an immense number of dead and dying chestnut trees on park lands under his supervision. Five years later, the first scientific bulletin appeared about the disease, a fungus later named *Endothia parasitica* (Murrill 1908; Gravatt 1930; Hepting 1974; Kulman 1978). Only a year after the bulletin's publication, an editorial in the *Southern Lumberman* referred to a "mysterious blight" that had recently been observed in Pennsylvania and New York. "Large timbered sections of [Pennsylvania] are already and in an alarming manner affected by the disease," stated the report (Southern Lumberman 1910). By 1912 all the chestnut trees in New York City were dead and the chestnut blight had reached no fewer than 10 states. Scientists in Pennsylvania launched a vigorous control program, which included burning dead trees, monitoring its advance, and spraying infected trees (Anagnostakis and Hillman 1992). This effort, a scientist later commented, was a little like using toy swords to battle an enemy equipped with atomic bombs. At the same time, foresters told the public that "the control and ultimate extermination of [the chestnut blight] will sooner or later become a real accomplishment" (Brown and Davis 1995).

The disease spread relentlessly southward, at an astounding rate of some fifty miles per year. Aided by woodsmen and loggers who carried it on their shoes and axes, the blight first entered North Carolina near Stokes and Surry counties about 1913 (Buttrick 1925). Shady Valley, in upper east Tennessee, was hit by 1915 (Cole 1990). By 1920 the American chestnut in the Great Smoky Mountains was ultimately doomed, though there were few visible signs of the blight there before 1925 (Brown 2001). In nearby Yancey County, North Carolina, nearly one in every ten chestnut trees was showing signs of the disease by 1925; in Buncombe County, one in five trees was dying from the blight at that time (Silver 2003). North Carolina lumbermen even used the imminently encroaching disease as a last-ditch effort to defeat the proposed Great Smoky Mountains National Park. "Certainly nothing could be more unsightly than the gaunt and naked trunks of these dead trees, standing like skeletons in every vista which the eye turns," they wrote in 1931 (Baxter 1931). By the mid-1930s, the blight had reached north Georgia, and by 1940 there was scarcely a tree in the entire Appalachian region that was not dead or showed signs of being severely infected with the disease (Exum 1992; Davis 2000).

CHESTNUT MEMORIES

Although few people alive today remember what the Appalachian forests looked like before the blight devastated the region, those who did witness the trees in their native splendor provide indisputable testimony to their significance to the mountain environment. "This is an unbelievable thing: how many chestnuts there were," remembered Paul Woody, who grew up near Cataloochee, North Carolina (Woody 1973). Gifford Pinchot himself recalled seeing chestnut stands with individual trees thirteen feet across and with crowns spreading more than 120 feet above the forest floor (Wheeler 1988; Davis 2000). Writing in the October 1915 issue of *American Forestry*, Samuel Detwiler noted that the "finest chestnut trees in the world are found in the southern Appalachian Mountains," adding that a tree with a diameter of seventeen feet had been found in Francis Cove, North Carolina (Detwiler 1915). Charles Grossman, one of the first rangers at the new Great Smoky Mountains National Park, recorded a chestnut tree 9 feet, 8 inches in diameter at a point six feet off the ground. "The hollow portion is so large that [an adult] could stand up in it," wrote Grossman soon after discovering it. "This hollow runs more than 50 feet up the trunk and at its narrowest point is not less than three feet. This must be the tree of which I heard. A man lost some stock during a snowstorm and later found them safe in a hollow chestnut tree" (Wheeler 1988; Davis 2000; Brown 2001).

Due to their abundance and enormous size, the American chestnut ranked as the most important wildlife plant in the eastern United States. The largest trees could produce ten bushels or more of nuts. Reports of

chestnuts four inches deep on the forest floor were not uncommon in many parts of the Appalachian mountains. Many of the wildlife species that mountain people thought of as game--squirrels, wild turkey, white-tailed deer, black bear, raccoon, and grouse--depended on these chestnuts as a major food source. "The worst thing that ever happened in this country was when the chestnut trees died," recalled Walter Cole of east Tennessee. "Turkeys disappeared, and the squirrels were not one-tenth as many as there were before...bears got fat on chestnuts, coons got fat on chestnuts, and the woods was filled with wild turkey...most all game ate chestnut..." (Cole 1965). Will Effler, who grew up on the West Fork of the Little River in what is today the Great Smoky Mountains, recalled shooting a wild turkey that contained no fewer than ninety-two chestnuts, "still in the hulls and undigested" in its swollen craw (Weals 1991). The former Cades Cove resident Maynard Ledbetter once remarked that "back when there were chestnuts, bear got so fat they couldn't run fast; now the poor bear run like a fox" (Ledbetter 1989).

Non-game animals were equally dependent on the chestnut, including several unique insect species that relied upon chestnut trees as their principal food course. Paul Opler, formerly of the U.S. Fish and Wildlife Service, has estimated that at least seven native moths became extinct in the southern Appalachians as a result of the chestnut blight (Opler 1978). The chestnut also slowed the recovery of wildlife populations already suffering from loss of habitat by logging operations. Biologist James M. Hill ascribes the slow recovery of deer, wild turkey, goshawks, Cooper's hawks, cougar, and bobcat in the mountains to habitat destruction directly caused by the chestnut blight (Hill 1993).

Of course, humans seasonally ate chestnuts too, making them an important dietary supplement when the trees dropped their nuts after the first major frost. Each October, children living in the mountains scooped up chestnuts by the sackful, often hanging their cloth bags on nails outside the kitchen door until December when the nuts would begin to get wormy. Smoky Mountain resident Alie Newman Maples remembered: "As a little girl, me and my brother Ray would take a sack or a pail and go out to the woods. Strong winds blew in the night, and we would pick up gallons of chestnuts under each tree" (Maples 1973; Brown 2001). Environmental historian Margaret Brown notes in her book *Wild East: A Biography of the Great Smoky Mountains*, that many mountain families routinely baked chestnuts in the kitchen fireplace, roasting them in dutch ovens. Among her most notable entries are the chestnut memories of Delce Mae Carver, who remembered sackfuls of chestnuts hanging on nails near the kitchen, ready to be baked over a warming fire. Johnny Manning, another Smoky Mountain resident who grew up in Greenbrier Cove, recalls as a child "trading pocketfuls of chestnuts for school tablets and pencils" (Brown 2001). For some Smoky mountain residents, the earnings from fall chestnut gathering was known as "shoe money," as the funds were used to purchase children's shoes before the coming winter (Brown and Davis 1992; Condon 1994).

Cherokees in Appalachia made even more use of the nut, which they frequently added to cornmeal dough that "was boiled or baked." Cherokees also used leaves from the tree to alleviate heart troubles, and the sprouts were sometimes made into an astringent tea to treat healing sores and wounds (Wigginton 1972; Stewart in press). All mountain families gathered many bushels of chestnuts, often taking them by wagon to urban markets. John McCaulley, whose family foraged for chestnuts in the Great Smoky Mountains around 1910, remembered seeing in one mountain cabin, a "hundred bushels of chestnuts, piled up there, and about four men packing off, every day." McCaulley himself recalls gathering as many as seven bushels of chestnuts in a single day's outing. These, he said, were taken to Knoxville on mules where they were sold for "four dollars a bushel" (Brown and Davis 1992). Chestnuts were also routinely shipped by rail to major cities on the eastern seaboard. In 1911, West Virginia reported that one railroad station alone shipped 155,000 lbs. of chestnuts to destinations along the train's northerly route (Giddings 1912; Kulman 1978).

Another historical use of chestnuts in the mountain region was food for hogs. Frederick Law Olmstead, in his travels through the Appalachians in 1854, reported that raising hogs was "remarkable fine" in the

mountains due to the large chestnut mast crop. He also noted that the swine of the region were of "superior taste" than those raised elsewhere in the South, a fact that made mountain pork a much sought after commodity (Olmsted 1860; Weals 1981). The huge annual mast production made woodland grazing possible, so for a month or two each fall, hogs ran loose in the woods to feast on the chestnuts littering the forest floor. Martha Wachacha, recalling the scene around her home in Cherokee, North Carolina, said "there were about a hundred pigs when I first moved here. Pigs and hogs were so fat. There was plenty of chestnuts back then" (Wachacha 1989). In late November, or as soon as the weather got cold enough, mountain residents rounded up the fattened hogs for slaughter. Martin Tipton recalled that "mountain people needed those chestnuts. They ate them themselves, of course, but they depended upon them to feed their hogs" (Brown and Davis 1994). Chestnut-flavored pork hung in the smokehouse all winter, where it continued to be the primary source of protein for most families. A Virginia farmer commenting on the role of chestnuts in mountain agriculture noted that it "didn't cost a cent to raise chestnuts or hogs in those days. It was a very inexpensive way to farm. The people had money and had meat on the table too" (Nash 1988).

As a building material, chestnut timber was unsurpassed. Chestnut wood was also highly rot-resistant, making it ideal for roofing shingles, telephone poles, ship masts, railroad ties and almost any other use requiring durable, long-lasting timbers. In 1909, the timber industry placed the total value of chestnut timber in the United States at more than 20 million dollars (Stewart 2005). Builders found chestnut wood to be remarkably insect-proof and weather resistant, so chestnut logs made the best fence rails, fence posts, and caskets. "Chestnut wood," as George Kulman wryly noted, "carried man from cradle to grave, in crib and coffin" (Kulman 1978). Seymour Calhoun, a full-blood Cherokee, added that "it was soft wood and worked good: you could split it" (Calhoun 1973). Chestnut trees grew so large that in one documented case, an entire cabin in the Great Smoky Mountains National Park was constructed from a single tree (Brown 2001). A valuable source of tannic acid used in the leather industry, chestnut bark and rough chestnut cordwood was another important source of income for mountain residents. In Tennessee alone, 50,000 cords of wood were cut yearly to supply those tanneries in operation before 1912. This "tanbark" or "acid wood," as it was called locally, was taken largely from trees already cut for other purposes or small defective trees that were not of nut-bearing age. Commercial operations were also heavily engaged in the harvesting of chestnut trees for tanbark and cordwood. One observer remarked in 1931 that even though chestnut timber was once cut by lumbermen for the bark alone, "very little waste of this kind is now noted" (Frothingham 1925).

As might be expected during the era of industrial logging, the blight did not slow the harvest of chestnut trees; in fact, the cutting actually increased after the initial introduction of the disease. In fact, most lumber barons were harvesting the largest chestnut trees even before the blight was officially observed in the mountain region. Early on, lumbermen even doubted the potential devastation of the disease, believing that the fast-going trees would eventually regenerate across the mountain landscape. Moreover, they knew that a chestnut tree was worth money dead or alive, since foresters soon determined that it was possible to manufacture lumber from standing dead chestnuts for up to ten years after the death of the tree. In fact, "wormy chestnut" lumber became much sought after by builders and furniture makers alike for many decades to come. For acid wood, the salvage period was even longer: Reuben Robertson, then president of the Champion Fibre, estimated that the company cut chestnut trees for pulp and tannin twenty years after the blight first arrived in North Carolina (Nelson and Gravitt 1929; Robertson 1959).

A WHOLE WORLD DYING

The abundance of dying chestnut trees was also responsible for the expansion and growth of the region's leather tanning industry. By 1930, there were no fewer than twenty-one chestnut-fueled plants in the southern Appalachians, producing over one-half of the U.S. supply of vegetable based tannins. Within a

decade, however, almost all evidence of chestnut trees had vanished from the mountains as the growing tanning industry, the "largest consumer of chestnut," had found ways to use every part of the tree. After 1940, with the development of synthetic replacements in the production of tannin, the demand for chestnut greatly diminished, leaving only a few ghost-white skeletons to stand lone sentry over the once great Appalachian forest. The dead and dying chestnut snags were painful reminders to mountaineers that the mountain landscape, including an entire way of life, was all but gone. "Man, I had the awfulest feeling about that as a child, to look back yonder and see those trees dying," recalled Joe Tribble, a native of eastern Kentucky. "I thought the whole world was going to die" (Hawkings 1993). A similar sentiment was echoed by Martin Tipton, who remembered that he and his dad used to come upon the skeletons of the trees on their many mountain walks. "Dad said it looked like a third of the mountain was dying" recalled Tipton (Brown and Davis 1994).

Mountain residents were right to mourn the lost of the American chestnut. The chestnut tree was possibly the single most importance natural resource of the Appalachians, providing inhabitants with food, shelter, and in the early twentieth century, a much needed cash income. Knott County, Kentucky native Verna Mae Sloan recalled that life without the chestnut tree was almost unthinkable. "At first we thought they would come back, we didn't know they were blighted out forever," she remembered. "But the chestnut tree was the most important tree we had. We needed those chestnuts" (Sloan, Pers. Comm., 1998). In fall and winter chestnuts could be boiled or roasted over an open fire or traded at the local stores for much needed supplies. Having "the greatest durability of available native woods," chestnut timber was made into long-lasting boards, posts, shingles, and split-rail fences. The tender and abundant sprouts could even be pulled from the ground and fed to cattle as fodder. As a wildlife food, the chestnut was unsurpassed, and helped to keep local game populations at their highest levels in recorded memory. In a memoir written shortly before his death, Shady Valley, Tennessee native William Cole summed up the extraordinary value of the tree to mountain residents. "A favorite outing for me and my friends was to go to the ball ground on Sunday to collect chestnuts," wrote Cole. "The chestnut tree was a great tree, chestnut wood was a great wood, and chestnuts a good food" (Cole 1990).

Sadly, the chestnut blight made it very unlikely that the Appalachian mountaineers would return to their more self-sufficient way of life. By the late 1930s, the mountaineer was more off the farmstead than on it, as the food and folkways of the region's inhabitants were beginning to conspicuously change. By the early 1940's mountain families were utilizing less buttermilk and more whole milk, less rye and wheat breads and more light breads, and consuming more processed sugar and less maple syrup and honey. While their were some dietary constants throughout the region, such as the consumption of cornbread and biscuits, the use of canned and other "store-bought" foods increased significantly during the first three decades of the twentieth century (Wheeler 1935). For those who remained exclusively farmers, the practice of crop monoculture became a much more common way to farm. Family size dropped by more than two individuals, from 10 family members per household in 1910 to 7.62 per household in 1934. Home building techniques changed as well. "Boxed" houses--that is, frameless structures made exclusively with sawn planks and boards--gradually replaced log cabins as residents working seasonally for lumber companies had less time, or the extra help, to build traditional log homes. The number of working outbuildings on the homestead also diminished, including the smokehouse, springhouse, and separate kitchen facility. Furniture was no longer home-made and looms and spinning wheels largely became a thing of the past (Black 1928). Needless to say, everything from architecture to social relations was altered by the separation of the mountain environment from the mountaineer.

In many ways, the death of the American chestnut symbolized the end of a waning, albeit arguably vital, subsistence culture in the Appalachians. The loss of the tree no doubt gave additional advantage to the forces of industrialization that were gaining a stronger and stronger foothold on the regional and local economy. No longer able to range hogs and cattle in the woodland commons, trap fish in free-flowing streams, or gather chestnuts on the hillsides, the rural mountaineer increasingly looked to the milltown

and urban center for economic salvation. The environmental abuse of the mountains, along with their permanent removal from the traditional land base, made it extremely difficult for mountaineers to continue a semi-agrarian, and intimately forest-dependent, way of life. With the death of the chestnut, an entire world did die, eliminating subsistence practices that had been viable in the Appalachian Mountains for more than four centuries.

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THE BACKCROSS BREEDING PROGRAM OF THE AMERICAN CHESTNUT FOUNDATION

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Abstract: The blight resistance of oriental chestnut trees is being backcrossed into American chestnut using traditional plant breeding techniques. Progeny are screened for blight resistance by direct inoculation with the blight fungus, when they are old enough to survive inoculation, which is 3 or 4 years for trees with intermediate levels of blight resistance, and 1 or 2 years for trees with high levels of blight resistance. Trees are grown using intensive horticultural techniques. Probably the most unusual aspect of this breeding program in comparison to similar programs for crop plants is the large acreages over which trees are grown, and the fact that the objective is recovery of a genetically diverse species rather than an improved cultivar. Highly blight resistant progeny have been recovered from intercrosses of straight F_1s, B_1s and B_2s, suggesting strongly that it should be possible to backcross blight resistance into American chestnut. Currently, two sources of blight resistance are being advanced to B_3-F_2. These are expected to begin producing progeny suitable for outplanting within 2 to 3 years.

INTRODUCTION

The American chestnut tree, *Castanea dentata* (Marsh.) Borkh., has been destroyed as a dominant forest tree by a canker disease, chestnut blight, incited by *Cryphonectria parasitica* (Murr.) Barr. The blight fungus was introduced into eastern North America around the turn of the 20th Century, probably in blight cankers on imported Japanese chestnut, *C. crenata* Sieb & Zucc., nursery stock (Metcalfe and Collins, 1909). By 1950, the disease had killed almost all of the large American chestnut trees throughout their range.

By 1930, when the American chestnut was thought to be doomed, attempts had begun to breed blight-resistant replacements. These attempts were abandoned, for the most part, around 1960, when no trees had been developed that combined the blight resistance of oriental chestnut trees with the large size of American chestnut trees (Jaynes, 1994).

In 1961, what later proved to be viruses (Hillman *et al.*, 2000) were found infecting *C. parasitica* (Grente, 1961). The infected strains had been isolated from blight cankers on European chestnut trees, *Castanea sativa* Mill., growing in Italy. The viruses reduced the virulence of the blight fungus enough that infected strains could no longer kill European chestnut trees. Additionally the viruses spread from one canker to another, resulting, apparently, in the protection of entire stands of European chestnut. When viruses were introduced into blight cankers on European chestnut in France, the disease there was ameliorated. This discovery led to efforts to control blight on American chestnut with these viruses, which continue today. To date, the results of this effort have not been entirely satisfactory (Anagnostakis, 1990).

In 1981, Charles Burnham proposed that the blight resistance of oriental chestnut trees, primarily Chinese chestnut, *Castanea mollissima* Blume, could be backcrossed into American chestnut. For American chestnut, this was a new method of plant breeding that had not been used in previous attempts to develop blight-resistant, timber-type chestnut trees. In 1983, The American Chestnut Foundation was established as a not-for-profit corporation to help fund work on Burnham's proposal (Burnham *et al.*, 1986). In 1989,

the foundation had accumulated sufficient resources to hire a part-time researcher at a new research farm in Meadowview, VA, in the heart of the range of the American chestnut tree.

Subsequent to 1989, the foundation has grown to the point where it is supporting a large breeding effort in Meadowview, with four full-time workers tending trees on three farms totaling 130 acres. Additional workers are employed in Asheville, NC and at Penn State University to assist volunteer breeding efforts at eleven state chapters. The administrative headquarters in Bennington, VT, also supports volunteer breeding efforts in CT and VT. The purpose of this paper is to describe progress to date in this breeding program.

MATERIALS AND METHODS

Breeding Method

To transfer blight resistance from Chinese to American chestnut, individuals of the two species are first crossed. The progeny from this cross, first hybrids, or F_1s, usually are exactly one-half American and one-half Chinese chestnut. An F_1 is backcrossed to another American chestnut, decreasing the proportion of Chinese chestnut genes by a factor of one half, on average. The progeny of this second cross, the first backcross, are known as B_1s. Two more backcrosses again decrease the proportion of Chinese chestnut genes by a factor of one half each time, to one-eighth followed by one-sixteenth, on average, with the remaining fraction of genes being from the American parent.

At each step of backcrossing, resistant trees are selected by observing canker symptoms after inoculation of the progeny with the chestnut blight fungus (see below for details). The progeny also vary in the fraction of Chinese genes remaining, and selection against Chinese morphological type is made to accelerate recovery of the American type, using traits identified by Hebard (1995). Burnham estimated that three backcrosses to the American parent, with selection against Chinese morphological type, would be sufficient to recover trees that look and grow like the American chestnut of old.

The F_1 trees, and any subsequent backcross progeny, would be heterozygous, at best, for the genes conferring blight resistance. Thus they would not be true breeding for blight resistance, throwing both susceptible as well as resistant progeny. To recover trees homozygous for blight resistance, third backcross trees are intercrossed among themselves, so the progeny have a chance of inheriting the genes for blight resistance from both parents. The progeny of this first intercross of third backcross trees are known as B_3-F_2s.

Blight resistance is only partially dominant, so F_1s and backcrosses are, at best, intermediate in resistance between the two parent species. High levels of blight resistance, comparable to those found in the Chinese parent, are only recovered after intercrossing F_1 hybrids and backcrosses. This facilitates recovery of trees reasonably homozygous for blight resistance, since they test out as more resistant than heterozygotes.

To avoid inbreeding, and its consequent decrease in genetic diversity, a different American chestnut parent is used at each step of backcrossing. Thus, in an ideal situation, four American parents are used to produce a third backcross tree. The third backcross progeny from a unique set of four American parents are termed a recurrent parent line or line for short. At the intercrossing stage, more than one line is needed in order to minimize sib crosses and their resulting inbreeding. Hebard (1993) estimated that 20 lines would be needed to minimize loss of alleles from inbreeding. With four American parents per line, 20 lines require 80 separate American parents.

In practice, only one line was used until the first backcross with the 'Graves' and 'Clapper' sources of blight resistance. These two first backcross trees then were crossed with 20 American parents to yield the second backcross generation, and with 20 additional parents to yield the third backcross. Thus the third backcross progeny are half first cousins rather than half third cousins.

To ensure that the progeny from intercrossing third backcross trees are homozygous for blight resistance loci, only one Chinese chestnut parent is used to make a set of 20 lines.

Sources of Blight Resistance

The availability of the named first backcross, 'Clapper' (Little and Diller, 1964), and the undescribed 'Graves' first backcross at the Connecticut Agricultural Research Station plantings in Hamden gave a jump start to the breeding program in 1989. These two first backcross trees were backcrossed again onto about 30 American chestnut trees each between 1989 and 1995 to yield second backcross trees, or B_2s. Thirty American chestnut lines of third backcrosses were produced between 1996 and 2003 for both the 'Clapper' and the 'Graves' lines. From 2001 until present, second generation third backcross progeny, or B_3-F_2s, have been collected and planted from intercrosses within sources of blight resistance. The Chinese chestnut grandparent of 'Graves' is an undescribed seedling known as 'Mahogany.'

In 1989, breeding also was started with the Chinese chestnut cultivar, Nanking, crossing it with 20 American chestnut trees to start 20 recurrent parent lines at F_1. Cultivar Nanking was chosen because it had shown the highest blight resistance of any Chinese chestnut tree evaluated by Headland and Griffin (1976) and was noted as having high blight resistance when first released.

As available, other Chinese and Japanese chestnut trees, and F_1 hybrids between these species and American chestnut, were crossed with American chestnut trees, in these later cases with only a few American chestnut trees rather than assembling 20 lines. Table 1 lists the sources of blight resistance at their most advanced stage of backcrossing as of April, 2004, and the number of American parent lines at the most advanced stage. As indicated above, additional lines occur at less advanced stages of backcrossing for some sources of blight resistance.

Table 1. Oriental sources of blight resistance being used at The American Chestnut Foundation's Research Farms in Meadowview, VA, their most advanced stage of backcrossing into American chestnut and the number of American parent lines at that stage as of April, 2004.

Source of Blight Resistance	**Stage of Backcrossing**	**Number of American Parent Lines**
Clapper	B_3-F_2	12
Mahogany	B_3-F_2	5
Douglas	B_3	2
Nanking	B_3	2
Sleeping Giant South Lot R11T14	B_3	1
Sleeping Giant South Lot R1T4	B_3	1
Sleeping Giant South Lot R1T7	B_3	3
Meiling	B_2	1
MusickChinese	B_2	2

Greg Miller 72-211	B_1	3
mollissima7	B_1	1
mollissima10	B_1	1
mollissima13	B_1	1
PI#104016	Japanese B_1	1
Dunstan seedling	F_1	1
FP7284	F_1	1
Greg Miller 65-18	F_1	3
Greg Miller 65-4	F_1	6
Kuling	F_1	4
Orrin	F_1	4
mollissima11	F_1	1
mollissima18	F_1	1
MAJ7Japanese	Japanese F_1	1
Jayne	mollissima x pumila	1
AbbsValley	Chinese	
Altamont	Chinese	
Armstrong	Chinese	
Eaton	Chinese	
MacBoyd	Chinese	
MAJ	Chinese	
MAJ4	Chinese	
MAJ5	Chinese	
Waynesboro	Chinese	
mollissima12	Chinese	
mollissima14	Chinese	
mollissima15	Chinese	
mollissima16	Chinese	
mollissima17	Chinese	
mollissima19	Chinese	
mollissima20	Chinese	
mollissima8	Chinese	
PI#7284	Chinese	
PI#97853	Chinese	
Richwood	Chinese	
Wilkinson	Chinese	
YardChinese	Chinese	
FPGlenDaleID:GS	Japanese	

American Chestnut Parents

In addition to the breeding at Meadowview, the American Chestnut Foundation also has an extensive network of state chapters staffed primarily by volunteers, and advised by staff officers stationed in North Carolina and Pennsylvania (Paul Sisco and Sara Fitzsimmons, respectively). The chapters have been crossing pollen of 'Graves' and 'Clapper' second backcrosses from Meadowview onto local American chestnut trees to produce third backcross trees, for the most part. The intent is to produce a viable breeding population of 20 individuals for each source of blight resistance, adapted to the local conditions, and also to increase the genetic diversity of the breeding population, as originally proposed by Inman (1987). Table 2 depicts the number of third backcross trees in the various states as of 2004.

Table 2. Number of third-backcross (B_3) chestnut at TACF breeding orchards in 2004, with the number of sources of blight resistance and the number of American chestnut lines in the breeding stock.

State	Nuts or Trees	Number of Sources of Resistance	American Lines
Maine	1445	2	29
Massachusetts	3076	2	28
Pennsylvania	5350	2	36
Maryland	33	1	1
Indiana	1496	1	11
Kentucky	150	2	2
Virginia (Meadowview)	5275	8	73
North & South Carolina	1049	2	9
Tennessee	745	5	6
Alabama	566	1	5
Total	**19179**		

Following Inman's recommendation (Inman, 1989), attempts have been made to limit the range of American chestnut parents to within 20 miles of each other in building local populations. This has been easier near Meadowview than elsewhere, since the required numbers of flowering chestnut trees can be found within such a small area.

Pollination

First hybrids and straight backcrosses are produced using the controlled pollination techniques described by Rutter (1991). Subsequent experience indicates that the best time to bag chestnut flowers for controlled pollination when the styles begin to emerge from the bur, rather than to assess the time by observing the onset of anthesis, as recommended by Rutter (1991). Experience also suggests that the slide technique using dried pollen described by Rutter (1991) to be more efficient than pollinating with fresh catkins. Flat surfaces other than microscope slides have been found preferable for applying pollen, such as the lid of the pollen container. In general, about one nut is produced per pollination bag placed over female flowers.

The intereross generations are produeed by open pollination, where possible. Thus breeding orehards containing straight third baekeross trees (B_3) from one sources of blight resistanee are isolated as mueh as possible from orehards with other sourees of blight resistanee or trees at other stages of breeding. Likewise, seed orehards, sueh as of B_3-F_2 trees, are isolated as mueh as possible from other orchards. A distanee between orehards of about 1 kilometer is estimated to be suffieient to isolate orehards. Pollen from undesired trees also is eliminated by emasculation, pruning at ground level and removal of the undesired trees.

Cultivation

The cultivation methods employed are standard orehard practiees adapted to screening ehestnut trees for blight resistanee. Hebard (1991) diseussed loeating flowering Ameriean ehestnut trees, and Hebard and Rutter (1991) outlined eultivation methods suitable for breeding orehards. Hebard (1994a) deseribed the teehniques for inoculating chestnut trees to test their blight resistanee, and the orehard spaeings used to grow trees. More reeently, Hebard presented designs for seed orehards and methods for produeing seed in them (2002) and methods for introdueing additional sourees of blight resistanee into our ehapter breeding programs (2001).

Orehards where baekeross progenies are to be sereened for blight resistanee are arranged in eompletely randomized designs with eontrols eonsisting of 6 to 12 individuals eaeh of pure Ameriean and pure Chinese ehestnut trees, and their F_1 hybrid. This experimental design was ehosen beeause eaeh genotype is unique, with no replieation of genotypes.

In a test of the response of trees of various ages to direet inoeulation, the intermediate blight resistanee of F_1 hybrids as young as 1 year old was distinguished from the high resistance of pure Chinese and from suseeptible pure Ameriean ehestnut trees. However, F_1 hybrids did not survive the test unless they were at least 3 years of age. Thus straight seeond baekerosses, whieh also have blight resistanee up to the intermediate level found in F_1 hybrids, are sereened for blight resistanee when they are 3 or 4 years old. At those ages and under our growing eondition, their diameter at breast height (1.5 m) ranges from 3 to 7.5 em (1 to 3 inehes) and their height from 3 to 5 m (10 to 15 feet).

In order to avoid erowding prior to blight resistanee sereening, trees to be sereened at 3 years of age are grown at a spacing of 1.2 m (4 feet) within rows. Trees screened for blight resistanee at 4 years of age are grown at a spacing of 2.1 m (7 feet) within rows. Originally, straight baekcross trees were sereened for blight resistanee at 4 years of age. Currently, straight baekeross trees are sereened for blight resistanee when they are 3 years old, except for third baekeross trees, whieh are sereened when 4 years old (we did not wish to ehange methods for our most valuable breeding material). Progeny of large, surviving Ameriean chestnut trees also are sereened for blight resistanee when they are 4 years old. To provide aceess for equipment, the between-row spacing in these orchards is 6 m (20 feet).

Progenies expeeted to contain blight-resistant individuals, sueh as F_2 generations, are sereened for blight resistanee when they are 1 or 2 years old. The blight-resistant progeny generally survive inoeulation at that young age. These are spaced within rows at 30 or 60 cm (1 or 2 feet). The between-row spaeing for F_2 progeny varies from 2.1 to 6 m (7 to 20 feet) depending upon the loeation and intent of the test.

Nuts are sown direetly at orehard spaeing. Prior to planting, orehard rows are subsoiled, plowed and rototilled, and 31.75-µm (1.25-mil) blaek plastic muleh lain in 1.22-m-wide (4 feet) strips. Using handled bulb planters, holes are drilled through the mulch into the soil and filled with a mix of one-third eaeh ground, milled peat moss, perlite and eoarse vermiculite. Nuts are planted 1-em deep (0.5 inches) and proteeted from voles with aluminum eylinders 25.4-em tall (10 inches) and 5 to 7 em wide (2 to 3 inches). After planting, the eylinders are jammed down around the nuts to a depth of about 5 em (2 inehes). The

aluminum is painted to reduce aluminum toxicity should it dissolve into the soil. Soil is mounded around the cylinders to prevent them from being blown away by wind. Styrofoam cups are inverted over cylinders until shoots emerge from the cylinders. At that point, the bottom of the cup is removed, and the cup replaced, to diminish breaking of the young shoots on the edge of the cylinders.

The seedlings generally outgrow the width of the cylinders during their third growing season. At the beginning of the third growing season, the cylinders are removed. The mulch also is removed to reduce vole damage. Prior to this time, the cylinders prevent vole damage. Voles can be harbored under mulch.

While black plastic mulch is in place, trees are fertilized with soluble fertilizer with a major nutrient composition of 30-10-10 (N-P-K) plus cationic trace elements (MirAcid™ or equivalent). Liquid fertilizer is used in order to place the fertilizer under the impermeable mulch. Approximately 2 liters (2 quarts) of fertilizer solution is applied every 2 weeks between mid May and early August. The fertilizer concentration is 3.26 ml per liter (1.25 tablespoons per gallon of water). Fertilizer is pumped directly down the cylinders or applied through a drip irrigation system. Once plastic mulch is removed, granular fertilizer is broadcast around the trees. The rate for granular fertilizer usually is 224 kg per hectare (200 lbs per acre) of N as ammonium nitrate and diammonium phosphate, 67 kg per hectare (60 lbs per acre) of P as diammonium phosphate and 67 kg per hectare of K as potash. These amounts are applied twice a year, in mid May and late June. In seed orchards, to avoid having to apply liquid fertilizer underneath plastic mulch, landscape fabric is used for mulching and granular fertilizer is broadcast at the above rates. The rates were formulated from soil and foliar mineral analysis for the soils typical of Meadowview and might differ on other soils. The rates also are adjusted depending upon the results of soil mineral analysis.

On trees 5 years of age and younger, weeds are managed with herbicides and mulch. In general, no weed management is performed on trees older than 5 years of age, other than mowing. Currently, in April, Surflan™ A.S. (oryzlin) is applied at 9.35 liters per hectare (4 quarts per acre), simizine 4L at 7.02 liters per hectare (3 quarts per acre) and Roundup Ultra™ (glyphosate) at 3.07 liters per hectare (42 oz per acre). A supplemental spray of Roundup Ultra™ at 3.07 liters per hectare (42 oz per acre) is applied in July to trees younger than 3 years old. These herbicides are applied as a directed spray using TeeJet™ 8005 standard flat-fan nozzles operated at 2.07 bars (30 psi) in a water solution of 608 liters per hectare (65 gallons per acre). The combination of low pressure with high volume spray nozzles increases droplet size, reducing drift. A strip 152.4 cm wide (3 nozzles at 50.8-cm or 20-inch spacing, 45.72 cm or 18 inches above the ground) is sprayed down each side of a row. The nozzle closest to the trees is directed with a hand wand, the other two nozzles are mounted on the boom of the spray rig.

Grass strips are maintained between rows to reduce erosion. Fire hazard is reduced by regular mowing with rotary cutters. In B_3-F_2 seedling seed orchards, which are sown at much higher densities (0.3 x 2.1 m, 1 x 7 feet), maintenance is performed with a riding lawn mower. Weeding of seedling seed orchards is done as above, but using a 25-gallon tow-behind sprayer attached to the lawn mower rather than a 65-gallon herbicide spray rig mounted on the three-point hitch of the standard orchard tractors used in the larger orchards. Only two nozzles are used in seedling seed orchards. The lawn mower-mounted nozzle is attached to the front of the mower. The mower operator also can manipulate a hand wand fairly easily on the lawn mower, whereas on the larger orchard tractors it is best if the hand nozzle is operated by a person walking behind. A pressure regulator needs to be added to most tow-behind sprayers. Their pumps are driven by electric motors powered from the lawn mower's electrical system, whereas the power take off drives the pumps on the orchard tractors. Thus it is important that the lawn mower produce enough electric current to power the pump.

Using an airblast sprayer, aphids are controlled with a single application of dormant oil during bud break at 56 liters per hectare (6 gallons per acre) in 2807 liters per hectare (300 gallons per acre) of water

solution. In July, Japanese beetles are controlled with 2 to 3 applications of Sevin XLR Plus™ at 5.8 liters per hectare (0.625 gallons per acre) in 935 liters per acre (100 gallons per acre) of water solution. Spray amounts have been reduced considerably by employing a Durand-Wayland Smart Spray 1000™ attached to a Durand-Wayland model AF500CPS airblast sprayer. This device cuts off banks of nozzles depending upon tree height and occurrence.

The pesticide application methods, composition, and rates were formulated in consultation with extension specialists from the Virginia Polytechnic Institute and State University and the "Spray Bulletin for Commercial Fruit Growers," which is issued annually (Virginia, West Virginia & Maryland Cooperative Extension Services, 2004).

Straight backcross trees have been irrigated in the year of inoculation during dry years. Since the year 2000, all young chestnut trees have been irrigated, except B_3-F_2 seedlings, using a drip irrigation system. Soil moisture is maintained at field capacity (about 10-20 kiloPascals of soil moisture deficit). We plqn to not irrigate B_3-F_2 seedling seed orchards.

Trees are not pruned for shaping or for removal of lower branches, as is often done in commercial fruit and nut orchards to facilitate passage down the rows and weeding with herbicides, among other objectives. Not pruning results in a crown that extends to the ground on the trees (and necessitates a second person walking behind the herbicide sprayer to prune off portions of branches that are sprayed inadvertently). This larger crown may promote early and heavier bearing. For the most part, our trees produce male catkins when they are 2 to 4 years old and bisexual catkins when they are 3 to 5 years old. This early flowering also has been seen in other hardwood trees grown under intense cultivation (Wright, 1976).

Using the above methods, the trees at Meadowview have averaged 0.56 m tall after one growing season, 1.5 m (5 feet) tall after two, 2.4 m (8 feet) after three, and 3.7 m (12 feet) after four growing seasons. There can be considerable variation in height growth within orchards and between growing season, genotype and location.

Screening for Blight Resistance

The cork-borer, agar-disk method is used to inoculate chestnut trees with the blight fungus (Griffin, *et al.* 1983). Agar disks are obtained from the margins of growing cultures that have not reached the edge of the Petri plate. Inoculations are performed in early June. This is the earliest in the season when cool weather (daily high temperatures below 15 to 20 C) can be avoided reliably. Cool weather occurs every few years in late May in Meadowview and can lead to inoculation failure.

Two strains of the blight fungus are used, known as Ep155 and SG1 2-3. Ep155 is a widely used strain of the blight fungus (ATCC 38755), while SG1 2-3 was isolated near Meadowview by the author. When tested for pathogenicity in American chestnut, the distribution of lengths of cankers incited by virulent strains of the blight fungus follows a bell-shaped curve; it is approximately normally distributed, and variances are equal for the various canker lengths (Griffin, et al, 1983). When replicated five times each over 3 years, or 15 total replicates, Ep 155 was among the most pathogenic of 21 tested virulent strains, having significantly ($p < 0.05$) larger cankers than six of the least pathogenic test strains. Likewise, SG1 2-3 was among the least pathogenic of the 21 tested strains, having significantly smaller cankers than seven of the most pathogenic test strains.

Blight resistance can be determined quantitatively by measuring the length and width of cankers. Canker depth or superficiality is not determined at Meadowview since the intermediate to very high levels of blight resistance being sought can be distinguished using length and width measurements alone. Until

1999, the length and width of cankers was measured on all tested trees. Because this was taking too much time, beginning in 1999, blight resistance in most tests was determined using a qualitative assessment.

The qualitative assessment is based on the following observations. In general, 1 year after inoculation, SG1 2-3 incites small cankers (2-3 cm long) on trees with intermediate levels of blight resistance or higher. It incites medium-sized cankers (3-6 cm long) on trees with low levels of blight resistance, and large cankers (> 6 cm long) on normal American chestnut trees. In contrast, Ep 155 incites large cankers on trees with intermediate levels of blight resistance or less, medium-sized cankers on trees with high levels of blight resistance, and small cankers on trees with very high levels of blight resistance. Thus five blight resistance classes can be distinguished on trees inoculated with both strains. This is depicted visually in Table 3.

Table 3. Blight resistance classes distinguished qualitatively by various canker length classes for two strains of *Cryphonectria parasitica* one year after inoculation in early June.

Numeric blight resistance class	Verbal blight resistance class	Length (cm) of canker incited by	
		Ep 155	SG1 2-3
1	highly blight resistant	2-3	2-3
2	blight resistant	3-6	2-3
3	intermediately blight resistant	> 6	2-3
4	slightly blight resistant	>> 6	3-6
5	not blight resistant or susceptible	>>> 6	>6

Table 3 depicts idealized canker lengths for various blight resistance classes seen in average years. Depending upon the season, slightly blight-resistant trees might show small SG1 2-3 cankers or blight-resistant trees might show large Ep 155 cankers. Additionally, the responses to the two strains do not always move in parallel with each other. These various unusual patterns of response can be detected by the response of the pure American and Chinese chestnut trees and their F_1 hybrids planted as control trees in the orchard and the scale adjusted accordingly.

In addition to artificial inoculation, trees in Meadowview also are exposed to naturally occurring inoculum. Blight incidence due to natural infections on straight backcross progeny exceeds 50% by the beginning of the fifth growing season, when trees are four years old. When screening artificially inoculated trees for blight resistance, the severity of these naturally occurring cankers is considered in the overall assessment of a tree. Thus, while only two strains of the blight fungus are used for direct inoculation, a larger number of strains is involved in the overall assessment.

RESULTS AND DISCUSSION

Recovery of highly blight-resistant backcross progeny at F_2

The first screening of progeny segregating for blight resistance in Meadowview occurred in 1993. One set of progeny consisted of B_1-F_2s obtained from reciprocal crosses of the 'Graves' and 'Clapper' trees. A second set of progeny consisted of straight F_2s obtained from a one-way cross of two F_1s. The F_1

parents were half sibs from crosses of the 'Mahogany' Chinese chestnut tree with pollen from two American chestnut trees. A third set of progeny segregating for blight resistance consisted of straight B_2s composited from three crosses of pollen from the 'Graves' tree onto three American chestnut trees. The trees were 2 years old when inoculated in June, 1993, and the data in Table 4 summarize canker dimensions when measured in September, 1993. Each tree was inoculated once with strain Ep 155 and once with strain SG1 2-3, using the cork borer, agar-disk method with holes 2 mm in diameter. Highly blight-resistant progeny were recovered from the F_2 and the B_1-F_2 crosses, and progeny with intermediate levels of blight resistance were recovered from the B_2 crosses. The B_1-F_2 crosses may have had higher blight resistance than the straight F_2s. Figure 1 depicts one of these highly blight-resistant B_1-F_2s.

Table 4. Mean and standard deviation and distribution of canker size classes (mean length and width of cankers incited by two strains of the blight fungus) for straight F_2, B_1-F_2 and B_2 American x Chinese chestnut progeny and controls.

Cross Type	Canker size class (cm)							Mean	Standard deviation
	1.0 to 2.6	2.6 to 4.2	4.2 to 5.8	5.8 to 7.4	7.4 to 9.0	9.0 to 10.6	10.6 to...		
Seedling American					3	5	2	9.6	1.1
F_1 'Nanking'				2	4	3		8.4	1.0
Seedling Chinese		2	7	3				5.2	1.0
'Meiling' Chinese		1	2	2				5.5	1.1
'Nanking' Chinese	3		2					2.9	1.4
F_2 'Mahogany'		5	23	48	48	29	15	7.7	1.9
B_1-F_2 'Clapper' x 'Graves'	4	25	84	116	112	54	4	6.9	1.9
B_2 'Graves'			2	4	15	26	6	9.1	1.5

Three-year-old B_2-F_2 progenies from controlled crosses between selected straight B_2s (backcrossed to American chestnut) were inoculated in June, 2003, and cankers measured in November. 'Clapper' B_2-F_2 progeny were from a single cross between two half sibs, while 'Graves' B_2-F_2 progeny were a composite of three crosses between half sibs. Depending upon their size, these trees were inoculated once or twice each with strains Ep 155 and SG1 2-3, using the cork borer, agar-disk method, but the holes were 4 mm in diameter. A larger cork borer and number of inoculations were used in 2003 than in 1993 because 2003's 3-year-old trees were larger than 1993's 2-year-old trees. Again, highly blight-resistant progeny were recovered, this time from second backcross F_2s (Table 5). Thus, not only could highly blight-resistant progeny be recovered by intercrossing F_1 interspecific hybrids or by intercrossing first or second backcrosses to American chestnut, but high levels of blight resistance were retained through the second backcross. These results suggest very strongly that the blight resistance of Chinese chestnut can be backcrossed into American chestnut.

Canker sizes were smaller in the 2003 than in the 1993 test, possibly because of cooler, wetter weather in the later year, so there was not as much separation of canker sizes among the controls. However, the cankers on some of the B_3-F_2 progeny have remained small through the 2004 growing season, as illustrated in Figure 2. An earlier test, performed in 1999 on open-pollinated progeny of 'Clapper' B_2s,

Figure 1. Highly blight-resistant Chinese to American B1-F2, 13 years old, 11 years after inoculation with *Cryphonectria parasitica.* The tree is to the left of and behind the dog.

presumably pollinated by other 'Clapper' B_2s, gave results similar to those presented in Table 5 (Hebard *et al*, 2000).

Blight resistance in straight backcrosses

Tables 6, 7, and 8 report typical results of rating straight second and third backcross trees for blight resistance. An entire family derived from a second backcross tree has not yet been rejected based on the performance of its third backcross progeny. In general, the blight resistance of third backcross progeny is comparable to that observed in second backcross trees, again supporting the inference that there is no diminution of resistance as backcrossing proceeds.

Family effects have occurred in second backcross progeny fathered by both the 'Graves' and 'Clapper' trees, where the American mother of second backcross progeny influenced their phenotypic blight resistance. This is illustrated in Table 9, where the Bu3C1C x 'Clapper' family had cankers closer in size to cankers on Chinese chestnut than on F_1s or Americans. It is unclear whether or not the Bu3C1C American parent was contributing genes for blight resistance by itself or contributing genes that

Table 5. Distribution of canker size classes (mean length and width of cankers incited by two strains of the blight fungus) for B_2-F_2 American x Chinese chestnut progeny and controls.

Cross Type	Canker size class (cm)							Mean	Standard deviation
	1.0 to 2.0	2.0 to 3.0	3.0 to 4.0	4.0 to 5.0	5.0 to 6.0	6.0 to 7.0	7.0 to 8.0		
Seedling American			4	2	2	2	1	5.0	1.4
F_1 'Nanking'		1	2	3	1			4.1	1.0
Seedling Chinese	3	3	3	6				3.3	1.2
B_2-F_2 'Clapper'	3	11	15	37	16	12	3	4.5	1.4
B_2-F_2 'Graves'	3	11	21	31	14	14	1	4.4	1.3

Table 6. Blight resistance ratings of 'Clapper' and 'Graves' second backcross trees and controls in 1999.

Cross type	Blight resistance rating				
	1	2	3	4	5
Seedling American				2	3
F_1 'Nanking'		4			
Seedling Chinese	3	5			
'Nanking' Chinese	1	1			
B_2 'Clapper'		5	27	29	12
B_2 'Graves'		3	42	47	25

Table 7. Blight resistance ratings of 'Clapper' third backcross trees and controls in 2000.

Cross type	Blight resistance rating				
	1	2	3	4	5
Seedling American				3	3
F_1 'Nanking'		2	10		
Seedling Chinese	3	2	1		
B_3 'Clapper'	1	19	139	383	95

Table 8. Blight resistance ratings of 'Graves' third backcross trees and controls in 2001.

Cross type	Blight resistance rating				
	1	2	3	4	5
Seedling American			1	3	8
F_1 'Nanking'		2	5		
Seedling Chinese	7	8			
B_3 'Graves'			124	124	122

modulated the expression of blight resistance genes from Chinese chestnut. The Bu3C1C tree itself did not appear to have more blight resistance than typical American chestnut trees; it died from blight the year after this cross was made, like most of the other American chestnut trees at that site.

Figure 2. Left, chestnut blight cankers after two growing seasons on a highly blight-resistant 'Clapper' B_2-F_2. Top left, canker incited by strain SG1 2-3. Bottom left, canker incited by strain Ep 155. Right, 4-year-old 'Clapper' B_2-F_2. Similar cankers on blight-susceptible American chestnut would be expected to exceed 40 cm in length; these cankers were 2 to 3 cm long.

Table 9. Distribution of canker size classes (mean length and width of cankers incited by two strains of the blight fungus) for progeny of second backcrosses of the 'Graves' and 'Clapper' first backcross trees to American chestnut and controls, in 1998.

Cross type	Canker size class (cm)						
	2 to 4	4 to 6	6 to 8	8 to 10	10 to 12	12 to 14	14 to 16
Seedling American					4	1	
F_1 'Nanking'			1	3	1		
Seedling Chinese		3	4				
'Nanking' Chinese	2	2					
Bu2B2C x 'Clapper'			2		3	2	
Bu2B3C x 'Clapper'			3	4	4	1	
Bu3C1C x 'Clapper'		15	33	8			
Bu1C1G x 'Graves'			4	8	17	10	1
Bu1C2G x 'Graves'			2	1	1	1	
Bu3B1G x 'Graves'				1			
Bu3B2G x 'Graves'					2		
Bu3C3C x 'Graves'		4	8	15	25	19	2
Bu3D1G x 'Graves'			1		2		
Bu3F1G x 'Graves'					1	1	
Bu3F5G x 'Graves'			2	2	5	2	
Bu3R1G x 'Graves'			2	7	13	4	1

Number of genes conditioning blight resistance

The standard deviations of canker size in Table 4 were greater for the progeny expected to be segregating for blight resistance than for the controls, and, for the F_2s, were compatible with models for one or two incompletely dominant genes controlling blight resistance, using Wright's method for estimating the number of factors controlling a segregating trait (Falconer, 1960, p 218). (In this computation, the total genetic variance of the F_2s was substituted for the additive genetic variance; the former was computed by subtracting the mean variance of the controls from the variance of the F_2s. The broad sense heritability calculated from these variances was about 70%). The distributions of canker size in segregating progeny in Table 4 were compatible with the distributions of canker size expected for two or three incompletely dominant genes of equal effect on blight resistance, among other models for gene action. Similar models with more than three factors or fewer than two did not fit the observed values (chi-square $p<<0.05$). The expected distributions were constructed from the mean response for the control trees, assuming a normal distribution of canker size with the average standard deviation of the controls shown in Table 4; missing cells, such as for trees with only one allele for resistance, were estimated by linear interpolation between the relevant observed values. Unfortunately, vegetatively propagated (grafted) individuals of 'Mahogany' were not available for inclusion in the test, nor the actual F_1 parents; otherwise stronger inferences might have been possible concerning the mode of inheritance of blight resistance. Subsequent experience suggests that 'Mahogany' has a high level of blight resistance, comparable to that of 'Nanking.' This

suggests in turn that two genes are involved in blight resistance. A three-gene model would be more compatible with the data if 'Mahogany' Chinese chestnut had a "normal" level of blight resistance like the 'Meiling' and seedling Chinese in Table 4 rather than the high level of blight resistance observed in 'Nanking.'Kubisiak, *et al.* (1997) prepared a genetic map of the 'Mahogany' F_2s whose canker sizes are shown in Table 4. Their results indicated that three regions of the genome were associated with blight resistance. The Kubisiak, *et al.* (1997) map was constructed with randomly amplified polymorphic deoxyribonucleic acid markers (RAPDs), restriction fragment length polymorphic markers (RFLPs), and isozymes. Subsequent genotyping of the mapping population with markers based on simple sequence repeats (SSRs) indicated that 17 of the 185 progeny were outcrosses, not pollinated by the supposed male parent (Sisco, Kubisiak and Hebard, unpublished). These individuals are not included in Table 4. One of the three regions of the genome previously associated with blight resistance (located on Kubisiak *et al.* (1997)'s linkage group G) was no longer associated with blight resistance in the revised mapping population. Molecular mapping of backcrosses of 'Mahogany' F_1s to American chestnut also suggested that the same two regions of the genome condition blight resistance (Kubisiak and Hebard, unpublished). The molecular mapping data thus supported a model of two incompletely dominant genes conditioning blight resistance in these progeny.

Highly blight resistant 'Clapper' x 'Graves' B_1-F_2 individuals were test crossed to American chestnut to determine whether or not they were homozygous for blight resistance. Screening of these 'Clapper' x 'Graves' test cross progenies indicated that they were segregating for blight resistance (data not shown), and hence that the B_1-F_2 parents were not homozygous. This finding suggests that some of the genes conditioning blight resistance in 'Clapper' and 'Graves' are at different loci. Highly blight-resistant 'Mahogany' F_2 progeny also had been test crossed to American. Unfortunately, all of the test-crossed individuals turned out to be outcrosses, as indicated by the SSR markers, invalidating this second set of tests.

There are numerous patterns of inheritance possible when a trait is controlled by more than one gene, including complementary inheritance, epistasis, etc (Grant, 1975). The model here of two incompletely dominant genes of equal effect is only one among these models, albeit one that fits the data. If further improvement of backcross chestnut trees for blight resistance is necessary beyond the B_3-F_2 stage of breeding, it might be best to use breeding methods for quantitative traits, such as recurrent selection.

The fact that the variance or range of canker sizes for the F_1 controls in Tables 4 to 9 were similar to those of the pure species indicates that 'Nanking' Chinese chestnut trees are homozygous for blight resistance. Similar data suggest that the named Chinese chestnut cultivars Orrin and Meiling, and the unnamed cultivars of Greg Miller, 64-4 and 72-211, likewise are homozygous for blight resistance.

Outbreeding and inbreeding depression

Not infrequently, specific Chinese x American chestnut crosses fail to produce nuts. Sometimes, nuts are produced, but fail to grow after germinating a radicle. These failures may be considered extreme instances of outbreeding depression. Chinese x American F_1 hybrids that do germinate often exceed pure species in size up to 10 to 20 years after planting, exhibiting hybrid vigor. For instance, after three seasons of growth, F_1 hybrids in four orchards were significantly ($p < 0.0001$) taller than pure species, having a least squares mean height of 2.2 m versus 1.8 m for the pure species. The F_1 hybrids also were significantly taller than any of the individual pure species.

The 'Mahogany' F_2s of Table 4 came from the only intercross of Chinese x American F_1 hybrids that has yielded well (greater than 1.0 nuts per pollination bag). Other F_1 intercrosses have yielded fewer than 0.6 nuts per pollination bag, sometimes much less. Attempts to use Chinese x American F_1 hybrids to pollinate American or Chinese chestnut trees also have produced low yields, in general. Even some

intercrosses among half-sib B_2s have yielded sound nuts that failed to produce seedlings. The failures of some of these more advanced crosses may be due to inbreeding depression rather than outbreeding depression. The failures (and pollen contamination in our early crosses) bedeviled attempts to repeat the early experiments. Similar failures also may have bedeviled attempts of earlier researchers to test hypotheses regarding the inheritance of chestnut blight resistance.

As mentioned previously in the section on blight resistance, the 'Clapper' x 'Graves' B_1-F_2s of Table 4 had more apparent blight resistance than the Mahogany F_2s. They also grew to be larger, more vigorous trees, perhaps because they did not suffer from inbreeding depression and/or had hybrid vigor (four-hundred, nineteen 'Clapper x Graves' and 'Graves x Clapper' progeny had a mean height at the end of the 1993 growing season of 2.43 m while 191 'Mahogany' F2s had a mean height of 2.13 m, significantly shorter, $p<0.0001$; a similar trend, $p=0.001$, was observed in 1992, prior to inoculation). The relative contributions of general vigor versus specific genes for blight resistance to the greater phenotypic blight resistance of the 'Clapper' x 'Graves' B_1-F_2s are unclear.

Summary

In sum, we have been able to recover highly blight-resistant chestnut trees after backcrossing blight resistance from Chinese into American chestnut for two cycles of backcrossing. Three cycles of backcrossing are expected to produce chestnut trees that, for the most part, look and grow like American chestnut. We currently are starting to test the blight resistance of second-generation, third backcross trees (B3-F2s), and currently expect some of them to have high levels of blight resistance. By 2008, we hope to begin planting their progeny (B3=F3s) back into the forest to confirm these expectations and to begin restoring the American chestnut tree to Appalachian forests.

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BLIGHT RESISTANCE TECHNOLOGY: TRANSGENIC APPROACHES

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Abstract: The technology needed to produce a blight-resistant, transgenic American chestnut tree has come to fruition. Many technical hurtles have been overcome and the first transgenic American chestnut trees are expected to be in the greenhouse within one to two years. These first trees will contain an oxalate oxidase transgene from wheat, under control of a regulated promoter from soybean. This will be the first of several putative resistance-enhancing transgene constructs to be tested for its ability to confer chestnut blight resistance. During the field trial phase of this research, we hope to include a few educational research plots accessible to the public under controlled conditions. In this way we can enhance the transparency of this project by allowing the general public to see first hand the results of this research. Once the transgenic trees are approved for release, they will enter a restoration program to rescue as much of the remaining genetic diversity in the surviving Ameriean chestnuts. This research should also help pave the way for the restoration of other threatened tree speeies through the use of biotechnology.

INTRODUCTION

Due to the movement of plant materials around the world, many exotic diseases and pests have threatened North American forests over the past century. Tree diseases such as white pine blister rust, chestnut blight, beech scale complex, Dutch-elm disease, butternut canker, dogwood anthracnose, just to name a few examples, have caused significant losses of trees in our forests and in urban settings. Although the United States has established several regulatory safeguards, diseases, such as the newly discovered sudden oak death, sometimes get through. It is likely that new introductions of diseases and pests will continue into the future. In an effort to restore species from past and ongoing epidemics, researchers are beginning to apply biotechnological techniques to forest species. In our labs, we are taking a transgenic approach to enhance pathogen resistance in American elm and in American chestnut. This report will describe the progress made to date and discuss the possible future of blight-resistant, transgenic American chestnut trees.

TRANSGENE DESIGN

When choosing transgenes to enhance pathogen resistance, and to play a vital role in the restoration of a valuable tree species such as the American chestnut (*Castanea dentata* (Marsh.) Borkh.), several considerations need to be taken into account. First, if the tree produces an eatable product, like the nut of the American chestnut, it must be equally as safe to eat for humans and wildlife as the nuts from non-transgenic trees. Second, the transgenic tree should have effective and durable resistanee to the blight. Third, the transgenic tree should retain all the positive traits of the American ehestnut so that it can be reestablished in its natural niche in the environment. Fourth, the transgenic tree should be amenable to a restoration program. For example, it should be able to recapture a significant portion of the remaining genetic diversity in the surviving population. Lastly, it needs to be acceptable to the general public, i.e.

the majority of the public should view the trees positively. In our transgene designs, we have considered all these aspects when choosing what to use.

Several genetic components needed to construct a variety of putative resistance enhancing genes are currently available (Powell et al. 1995; Powell and Maynard 1997; Powell et al. 2000; Liang et al. 2001; Connors et al. 2002; Connors et al. 2002; Liang et al. 2002). More putative resistance enhancing genes are being reported each year and someday the resistance genes from the Asian chestnuts trees might be cloned, enhanced, and used to transform American chestnut trees. But to save time and space, this report will focus only on the transgene construct that will be used first to produce a transgenic American chestnut. If this construct fulfills all the necessary criteria for producing a blight-resistant American chestnut, then this might be the only construct needed, but if this transgenic construct is not as effective as needed, many other genes and gene promoters are available. The first transgenic American chestnuts will contain a three-gene cassette as show in the binary vector pVSPB-OxO (fig. 1).

This construct contains an oxalate oxidase (OxO) encoding gene from wheat (Lane et al. 1986; Lane 1994). This gene was selected because it comes from a familiar plant that is consumed by most Americans every day and therefore brings with it a sense of public acceptability. It is also being researched for use to enhance pathogen resistance in other transgenic crop species, which should help with the regulatory approval as government reviewers become familiar with this transgene's properties. Lastly, its mechanism for enhancing resistance appears to be well suited for producing effective and durable resistance to the chestnut blight.

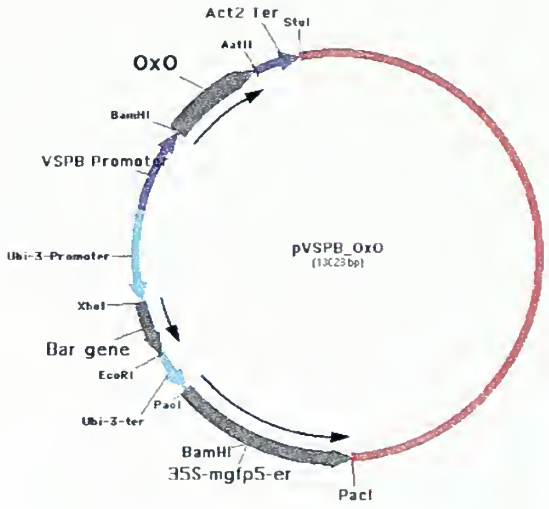

Figure 1. Plasmid map of the binary vector pVSPB-OxO.

Oxalate oxidase catalyzes the degradation of oxalic acid into H_2O_2 and CO_2. Similar enzymes have been found in several plant species such as barley and wheat and are expressed during germination, stress, and fungal infection (Dumas et al. 1995; Zhang et al. 1995; Hurkman and Tanaka 1996; Hurkman and Tanaka 1996). This enzyme is of interest because *Cryphonectria parasitica*, the chestnut blight fungus, produces large amounts of oxalate at the canker margin, which helps lower the pH to toxic levels and binds calcium (Roane et al. 1986). Oxalic acid, or oxalate, has also been associated with pathogenesis in other fungi (Noyes and Hancock 1981; Marciano et al. 1983; Cessna et al. 2000). Therefore, it is reasonable to hypothesize that neutralizing oxalic acid will enhance resistance to these fungi. One byproduct of the oxalate degradation, H_2O_2, could induce a separate defense mechanism, which would only be activated when the fungus produces oxalate. H_2O_2 has been shown to be a signal molecule that induces a plant's natural defense system (Lane et al. 1993; Lane 1994) and could enhance resistance in transgenic plants (Wu et al. 1995). Previously, we had cloned the wheat oxalate oxidase transgene into a model tree species, hybrid poplar, and shown that it could enhance resistance to another oxalate producing pathogen, *Septoria musiva* (Liang et al. 2001). Other researchers have cloned this same gene into soybean and shown enhanced resistance to the white mold fungus, *Sclerotinia sclerotiorum* (Cober et al. 2003). Recently, transgenic callus from American chestnut expressing the oxalate oxidase transgene and grown in the presence of oxalic acid, was shown to retain its ability to produce lignin at normal levels. Oxalic acid would significantly inhibit lignin formation in the non-transformed controls (Welch, Stipanovic, Maynard, and Powell, unpublished). Lignin synthesis is necessary to compartmentalize fungal infections in plants. Therefore, since the wheat oxalate oxidase

gene will have multiple resistance enhancing effects, we believe it will likely enhance blight resistance in American chestnut and be sustainable.

Attached to the oxalate oxidase transgene is a regulated promoter that can control which tissues in the plant can express the gene. In this construct, the promoter from the soybean vegetative storage protein B (VSPB) gene was chosen because its expression is induced by sucrose and by wounding and repressed by auxins (Mason et al. 1993; DeWald et al. 1994). Its expression pattern therefore is primarily in the stems and wound sites, the tissues that can be infected by the chestnut blight, and it is not expected to be produced in significant amounts in the nuts. The expression of the oxalate oxidase transgene in pVSPB-OxO has been tested in *Arabidopsis* and shows vascular expression as expected (fig. 2).

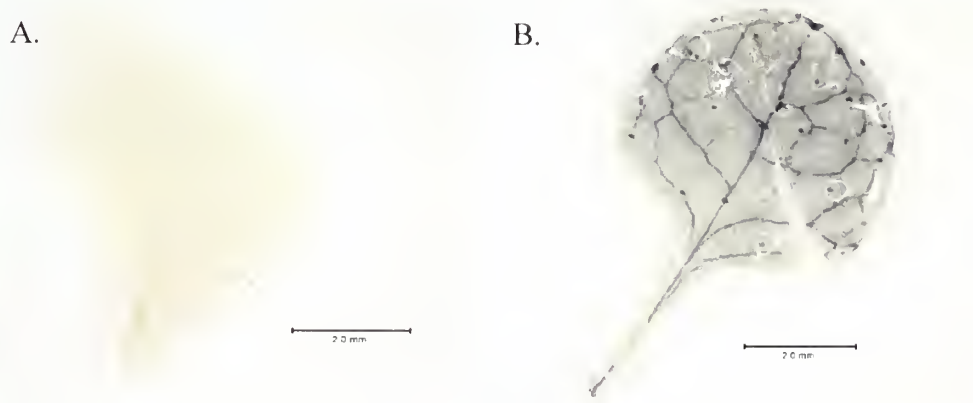

Figure 2. Oxalate oxidase assays of non-transformed Arabidopsis (A) and Arabidopsis transformed with pVSPB-OxO (B). The dark coloration in the vascular tissues seen in B is a positive result.

In addition to the putative resistance-enhancing gene, there are two other transgenes commonly found in binary vectors. The first is the BAR gene (Figueira Filho et al. 1994; Metz et al. 1998), which confers specific resistance to phosphinothricin (PPT), the active ingredient in the herbicide Finale®. In plant transformations, this gene is used for selection of transformed cells. In an American chestnut restoration project, this gene will also have a second useful function. To save as much of the genetic diversity, including rare alleles, in the surviving population of American chestnuts, a restoration program would need to out-cross to as many of these trees as possible. To do this efficiently, the transgenes must convey resistance in the hemizygous state, meaning full resistance from a single copy. If a hemizygous tree were to be out-crossed to a non-transgenic tree, only half of the resulting seeds would contain the transgenic resistance cassette. This is where the herbicide resistance would be useful. Nurserymen could plant all the seeds and as they sprout, spray them with Finale®. Only the transgenic trees would survive. This would save a lot of labor and money, and would increase the efficiency of a restoration program. This would also allow easy identification of the transgenic trees in the field by a technique called spotting. In this technique, a small drop of Finale® is spotted on a freshly excised leaf. If the plant is transgenic, no necrotic spot will appear, but after a few days, the non-transgenic plants will display a necrotic spot on the leaf.

Lastly, in pVSPB-OxO there is a gene encoding a green fluorescent protein (Haseloff et al. 1997). When illuminated by ultraviolet or blue light, this protein will fluoresce green and can be detected using specific filters. This gene has greatly enhanced our ability to optimize the transformation procedure because it allows visual identification of the transformed tissues without damaging them. This gene is currently being used to optimize our transformation procedures but may or may not be in the final transgenic tree released. Although this gene is harmless, we will gage public acceptance before using it in the released trees.

CHESTNUT SOMATIC EMBRYO TRANSFORMATIONS

This year, 2004, marks one hundred years since the discovery of the chestnut blight (Merkel 1906). This is also the year in which a method has been developed that can consistently produce transgenic American chestnut. Over the past fourteen years, foundation research has been accomplished in Dr. Maynard's and Dr. Merkle's labs (Merkle 1991; Carraway et al. 1997; Xing et al. 1997; Xing et al. 1999). This year, two advances have greatly improved the transformation protocol. The first is the use of GFP to identify and follow transformed tissues. The second was to add a desiccation step to the transformation procedure (Cheng 2003). To date, nine lab members and students have been able to transform chestnut embryos. Some of the best looking transgenic embryos are beginning to develop (fig. 3). Currently, the first transgenic lines of embryos are being propagated and a portion will be stored cryogenically in Dr. Merkle's lab (Holliday 2000). The remainder will begin the process of regeneration into whole plants.

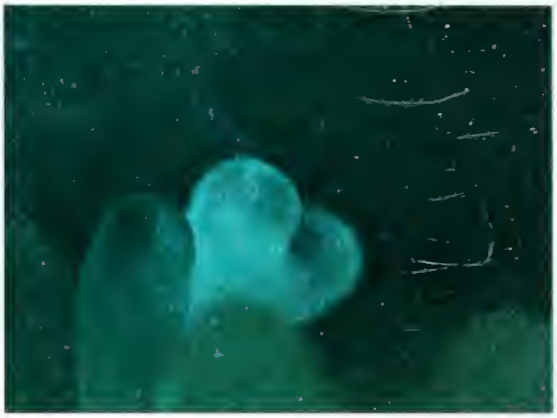

Figure 3. Example of an American chestnut somatic embryo transformed with pVSPB-OxO expressing GFP (transformed & photographed by Ron Rothrock, Dr. Maynard's graduate student).

NEXT STEPS

In parallel with the transformation work, we have been conducting studies on propagating American chestnut somatic-embryo-derived and apical-meristem-derived plantlets and acclimatizing those plantlets for establishment in the field (LaPierre 2003). The first non-transgenic, micropropagated American chestnut plantlets were established in a bare-root nursery in 1997 and were lifted, examined for root morphology (fig. 4), and transplanted to the field in 2001.

The next step in the process of evaluating the new transgenic somatic embryos will be to first multiply and germinate individual embryos (Merkle et al. 1991, Carraway et al. 1997; Xing et al. 1999), or if germination is low, micropropagate them (Xing et al. 1997). Once a sufficient supply of transgenic plantlets has been produced, they will be acclimatized and grown in the greenhouse (Biekel *et al* 2000) until they have a minimum stem caliper of 3 mm and then screened for transgene expression in the stem tissues. The trees that express the transgene as expected will then be tested for blight resistance. Those transformation events producing trees with a high level of blight resistance will be planted in field trials and evaluated against non-transgenic American and Chinese chestnut (*Castanea mollissima*) seedlings for resistance, growth characteristics, and mycorrhizal interactions.

Figure 4. American chestnut tissue-culture-derived trees (left of meter stick) and seedling controls (right of meter stick) after four growing seasons.

CONCLUSIONS

If all goes according to our projected timeline, we expect to have the first potted transgenic American chestnut trees in the greenhouse by the spring of 2005. At this time we will start the regulatory process for approved release, beginning with USDA notification of the field trials. These plants will then be hardened off and should be ready for field planting in the fall of 2005. We hope that some of the field trials can be set up as public educational demonstrations. These plantings will be in controlled areas, but will be accessible to the public so that they can observe the ongoing experiments and learn about the chestnut blight and about possible uses of forest biotechnology. The final transgene make-up of the transgenic American chestnut that will be released to the public will depend on the results from the resistance assays, field trials, regulatory approval, and public acceptance. Once approved for release, we believe that transgenic American chestnuts trees will play a key roll in the restoration of this threatened species.

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HYPOVIRULENCE: USE AND LIMITATIONS AS A CHESTNUT BLIGHT BIOLOGICAL CONTROL

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Abstract: The recovery of chestnut from chestnut blight in Italy and Michigan largely was responsible for the resurgence in chestnut research. The observed remission of disease now has been attributed to a biological control process called hypovirulence, whereby virulent strains are debilitated as a result of infection by fungal viruses (hypoviruses). Several species of hypoviruses now are known and each may impart unique effects on *Cryphonectria parasitica*. Lethal infections often are controlled by introducing the appropriate hypovirus into cankers. Unfortunately, at many locations within the native range of American chestnut, a complex system of vegetative incompatibility restricts hypovirus transmission among strains. Factors like vegetative incompatibility apparently regulate the widespread establishment of hypoviruses and presumably are, in part, responsible for our inability to artificially establish hypoviruses to the extent that has occurred naturally. Some of the factors that regulate hypovirus success or failure may be discovered as part of ongoing research at an isolated Wisconsin chestnut stand. Hopefully, understanding the phenomenon of hypovirulence eventually will allow it to be employed as part of the American chestnut restoration program.

INTRODUCTION

Cryphonectria parasitica was first recognized one hundred years ago as the fungus responsible for the cankers that resulted in the death of American chestnut (Merkel 1905). This brightly orange-pigmented organism was new to North America and by the time it was identified, its role in the tragedy that was about to unfold was cast. The remarkably detailed work of several early scientists unraveled the biological details of a host-pathogen relationship that would have unparalleled ecological and sociological impact (Anderson and Babcock 1913, Heald and Gardner 1913, Shear and Stevens 1913). Within ten years of the identification of *C. parasitica* as the causal fungus, their writings sadly were predictive of what was to ensue. As the blight spread, most of the research efforts turned to the strategy of breeding blight resistant trees (Fleet 1914). The early breeding programs met with limited success and never were designed to control blight in eastern forests. For almost fifty years, relatively little research attention was directed toward the causal fungus. If this same organism was introduced today, American chestnut undoubtedly would face the same fate.

We are fortunate that American chestnut was saved from extinction in its natural range by its propensity to sprout. The first one hundred years of chestnut blight is a blink in biological time. It may, however, be this surviving sprout population that over longer biological time periods allows for the expression of a disease of *C. parasitica* that may result in natural biological control of chestnut blight. A glimmer of hope that this was possible emerged from observations of "spontaneously healing" cankers that were noted on European chestnut growing in northern Italy (Grente 1965). The "recovery" phenomenon was confirmed when Jean Grente, a French mycologist, described a variety of unusual strains of *C. parasitica* that he isolated from the callusing cankers (Grente and Berthelay-Sauret 1978). The isolates he recovered often were lightly pigmented in contrast to the normal orange pigmented lethal strains. Moreover, Grente found that, like the infection from which they were isolated, when the isolates were inoculated to healthy

chestnuts, lethal infections seldom resulted. Most significant was the observation that the cause of this debilitation was transmissible to virulent strains. Grente coined the term "hypovirulent" to describe the reduced state of virulence and suggested that "cytoplasmic agents" were responsible for this phenomenon (Grente 1965). Remarkably, for the chestnut growing in northern Italy, this marked "recovery" was occurring within twenty-five years of the discovery of chestnut blight in Europe (Mittempergher 1978).

By the time recovery was detected in Italy, the spread of the chestnut blight fungus through the natural range of American chestnut was complete. There were few, if any, signs of resistance or recovery from infection. Grente's discovery and his research describing hypovirulence refocused attention on chestnut blight in this country, especially in the laboratories at The Connecticut Agricultural Experiment Station where a longstanding chestnut breeding program still was active. Further, the phenomenon of hypovirulence brought attention to the pathogen. It also was during this period of the early 1970s that a small stand of blighted chestnuts growing in Michigan was brought to the attention of the Connecticut research team (Elliston et al. 1977). They quickly discovered that the isolates from Michigan also carried a "cytoplasmic agent" that was transmissible and reduced the ability of *C. parasitica* to produce lethal cankers (Elliston 1985). Unlike the hypovirulent strains from Italy, the Michigan isolates retained their bright orange pigmentation. As the search for chestnut trees that were recovering from blight in Michigan intensified, more and more "recovering stands" were discovered, all outside the natural range of chestnut. Although chestnut blight is still the dominant stressing agent in most of the isolated Michigan stands, in some, the impact of the disease is minor, and the level of recovery mimics the expression of hypovirulence in some areas of Italy (Fulbright et al. 1983).

Since these two remarkable settings have been described, hypovirulent strains have been identified at various locations within the natural range of American chestnut and some are associated with surviving trees (Griffin 2000). Unfortunately, the wide-spread recovery of chestnut as a result of the hypovirulence phenomenon is unknown in areas where sprout populations still persist. Several reviews of the recovery of chestnut blight and associated hypovirulence are published (Milgroom and Cortesi 2004, MacDonald and Fulbright 1991, Heiniger and Rigling 1994, Nuss 1992, Van Alfen 1992).

WHAT IS HYPOVIRULENCE?

A variety of factors control the level of virulence in *C. parasitica* including the genetic makeup of the fungus or a variety of nonviral, cytoplasmic agents, such as defective mitochondria or plasmids. However, the term hypovirulence most often refers to the reduction in virulence caused by fungal viruses. The first indication that virus-like agents might be involved came with the association of double-stranded (ds) RNA with the European and North American strains that were shown to be less virulent (Day et al. 1977). These dsRNAs eventually were shown to represent a unique group of viruses, now called hypoviruses (Hillman et al. 2000). The definitive proof of the cause and effect relationship and their infectious nature occurred through the application of molecular technology (Choi and Nuss 1992). Although fungal-virus associations have been know for decades, the hypoviruses associated with *C. parasitica* are unique; rather than being encapsulated in a protein coat, they are membrane bounded (Newhouse et al. 1983). As a result, a new virus family, the *Hypoviridae*, has been established for the four species (CHV1 though CHV4) of hypoviruses that have been discovered to date (Hillman and Suzuki 2004). Most studies have been of the CHV1 species, as it was the first hypovirus identified and is the hypovirus associated with biological control of chestnut blight in Europe (Shapira et al. 1991). This hypovirus also has been discovered infecting strains of *C. parasitica* in China and Japan, but it has never been identified as a natural component of *C. parasitica* in North America (Peever et al. 1998). The CHV3 hypovirus is associated with the recovering Michigan chestnut stands but its origin remains unknown as it has not been isolated in the Orient (Paul and Fulbright 1988). CHV2 is uncommon and known only from a site in New Jersey (Hillman et al. 1994). It also has been identified in *C. parasitica*

populations in China (Peever et al. 1998). CHV4 is somewhat unique; unlike CHV1-CHV3, it has little or no observable effect on the virulence or other traits of *C. parasitica* (Enebak et al. 1994). It is widespread in its association with isolates from the central Appalachians but its origin and role remain undiscovered.

The effects of hypovirus infection on the blight fungus are variable and appear to be a function of the *C. parasitica* strain as well as the infecting hypovirus (Chen and Nuss 1999, MacDonald and Double 1998). For those hypoviruses that reduce fungal virulence, infection often results in smaller non-lethal cankers and a corresponding reduction in the production of asexual spores and almost certainly the reduction or elimination of sexual sporulation. What currently is known about the molecular influence of the hypovirus on the physiological processes of the fungus has been reviewed (Nuss 1992, Nuss 1996).

EXPLOITING HYPOVIRULENCE

The discovery of hypovirulence and the observation of a notable level of disease control on American chestnut in Michigan brought hopes for the first time that some level of biological control was possible in North America. Procedures first employed by Grente to treat virulent infections were duplicated. Subsequently, modifications to Grente's treatment protocols and a variety of different inoculum types were used to introduce hypoviruses into virulent cankers on American chestnut sprouts (Hobbins et al. 1992, MacDonald and Double 1979). The results often were very encouraging as hypovirus transfer frequently occurred and the expansion of individual treated infections frequently was arrested as callus tissue formed at the margins of cankers. Even though many of the treatments were successful and the life of sprouts was prolonged, the sheer number of subsequent infections that developed on the same stem dramatically weakened the tree, and when some cankers were not arrested by treatment, trees died (MacDonald and Fulbright 1998). Further, there was little evidence that natural hypovirus spread on the same stem afforded any protection to other virulent infections that almost certainly would arise. With few exceptions, most hypovirulent introductions were unsuccessful if measured by the number of treated sprouts that remained alive several years after treatment (Milgroom and Cortesi 2004)).

As a result of the early releases, several factors were discovered that may influence the effectiveness of the hypovirulent treatments. When additional hypovirulent strains were discovered and their infecting hypoviruses investigated, the variation in their effects on *C. parasitica* became apparent. Some virulent strains were so debilitated by hypovirus infection that they grew poorly in bark and almost completely failed to produce hypovirulent inoculum (Double and MacDonald 1995). Therefore, concern arose that highly debilitating hypoviruses have such an extreme effect on their fungus host that there is little potential for the strains to grow in bark and produce inoculum to perpetuate themselves. A sense developed that hypoviruses that do not debilitate *C. parasitica* as significantly may be more useful biological control agents (MacDonald and Fulbright 1998). Logically, if strains are more capable of invading bark and generating hypovirulent inoculum without killing their hosts, they may be more capable of disseminating their hypoviruses and thus potentially better biological control agents.

ROLE OF VEGETATIVE COMPATIBILITY

Early laboratory and field experimentation also revealed that an incompatibility system existed in *C. parasitica* (Anagnostakis 1977). When strains are incompatible, their hyphal elements fail to fuse (anastomose), restricting cytoplasmic and hypovirus exchange. Unlike many plant and animal viruses, viruses that infect fungi have no extracellular phase and therefore must be transmitted to progeny in spores during reproduction or via hyphal anastomosis and cytoplasmic mixing. The system of vegetative compatibility in *C. parasitica*, as in other fungi, represents a self-recognition system that prevents

incompatible strains from fusing. Essentially, as the hyphal filaments of the fungus approach each other, cell death occurs, restricting the fusions necessary for hypovirus transmission. The system of vegetative incompatibility in *C. parasitica* is controlled by at least six genes (Huber 1996, Cortesi and Milgroom 1998). The probability of hypovirus transmission is high when strains share identical genes. Transmission is less likely if gene differences exist with probabilities of transmission related to the number of gene differences and the specific genes present.

Considerable research on vegetative compatibility has been conducted (Cortesi and Milgroom 1998, Milgroom 1995, Milgroom and Cortesi 1999). One interesting relationship that has been discovered relates to the diversity of vegetative compatibility types and hypovirus transmission in field settings. In general, sites where biological control generally is more successful have a less diverse population of vegetative incompatibility genes (Milgroom et al. 1996). This appears to be the situation in Italy and Michigan (Cortesi et al. 1996) where the number of vegetative compatibility genes that are expressed is quite low when compared to the diversity that occurs in Asia or the central Appalachian region (Figure 1). Whether the lack of diversity is responsible for the widespread distribution of hypoviruses and biological control that has occurred is unknown.

Table 1. Diversity of vegetative compatibility types in four chestnut areas (two in the U.S. from *Castanea dentata* stands, one in Italy from a *C. sativa* stand, one in China from a *C. mollissima* stand and one in Japan from a *C. crenata* stand).

Population	Number of isolates tested	Number of VC types
Finzel, MD	57	25
Bartow, WV	61	29
Italy*	716	20
China*	79	71
Japan*	30	29

*Data from Milgroom

A second interesting relationship between hypovirus infection and the diversity of vegetative compatibility types is the effect hypovirus infection may have on the diversity of vegetative compatibility types at a site. Sexual reproduction is responsible for maintaining diversity (Marra and Milgroom 2001). One must therefore consider whether the low diversity that exists at some recovering sites is because hypovirus infections have reduced sexual reproduction or the reduced diversity has been a longstanding feature of the stand and has permitted hypoviruses to be disseminated successfully.

Although vegetative compatibility diversity appears to influence the success of biological control, other factors also may be involved. Certainly the role of the host cannot be overlooked in the expression of the hypovirulence phenomenon. In tests of susceptibility, European chestnuts consistently have been shown to be slightly more resistant than American chestnut (Bazzigher 1981). A more resistant host almost certainly would provide a longer time period for infections to acquire hypoviruses, perhaps enough time for the successful expression of the hypovirulence. Similarly, the ecosystems in which the two species typically grow are quite different. In its North American range, chestnut grows among a diverse mix of competing hardwoods. This often is not the case in Europe, especially in areas where European chestnut is cultivated for nut or coppice production (Bounous 1999). Likewise, at many recovering sites in Michigan, chestnuts grow in the absence of significant competition from other species. These settings permit the constant regeneration of chestnut biomass and may in turn foster the dissemination of hypoviruses. Unfortunately, in eastern North America, chestnut is largely a relic in the understory with little opportunity to grow and develop significant numbers of canker to even acquire hypoviruses. One

site where many of these limitations do not exist is in a stand of Ameriean ehestnut growing near West Salem, Wisconsin.

UTILIZING HYPOVIRUSES AT WEST SALEM

The West Salem stand is the largest stand of Ameriean chestnut in the U.S. The origin of the stand dates to the late 1800s when a few ehestnuts were planted at the site by the landowner who had moved there from the eastern U.S. (Cummings Carlson et al. 2002). Chestnut now is the dominant speeies on about 50 aeres of land. Unfortunately, in 1986, *C. parasitica* was diseovered at the site and now threatens the future of this magnifieent stand. Attempts from 1988-90 to eradieate the fungus failed. A biologieal eontrol program was initiated in 1992. At that time, the stand seemed to present an exeellent opportunity to exploit hypoviruses for two reasons. First, there were few trees infeeted so the disease was at the very early stage of the epidemic. Seeond, the stand was infeeted by a single elone of *C. parasitica*; henee, the barriers imposed by vegetative eompatibility did not exist (MeGuire and Milgroom 2002). Over the next six years, two hypoviruses were introdueed into the resident West Salem strain (Double and MaeDonald 2002). These were deployed by introdueing inoeulum into small holes made around the margin of the eanker. The first hypovirus (CHV3 type) deployed (1992-94) was obtained from a reeovering grove of ehestnuts near Cadillae, Michigan. The seeond was a hypovirus (CHV1 type) assoeiated with an Italian hypovirulent strain and was used for treatment from 1995-97. As eankers were treated, they routinely aequired hypovirus and the treated ehestnuts responded by producing signifieant eallus growth to elose the infeetion.

Between 1992-1997, about 650 eankers on 138 trees were treated. To assess hypovirus spread each season, 8-12 small bark plugs were removed from the treated eankers and also from new eankers that had formed. An evaluation of hypovirus infection was made when the plugs were eultured and the eultural appearance of the resulting colonies was eompared to that of virulent strains.

From 1998-2002, no additional hypovirus introduetions were made, so that an evaluation of the level of natural spread could be made over several seasons. The results have been mixed. Treated eankers have retained hypoviruses and many are heavily eallused and blight is no longer damaging (Table 1). Likewise, new eankers that have developed on trees with treated eankers have acquired hypovirus at high levels. Many of these stems are still alive almost ten years after initial treatment. Unfortunately, hypoviruses have not spread signifieantly to eankers on nearby trees that never reeeived hypovirus treatment (Double and MaeDonald 2002). Beeause the number of new virulent infections eontinues to increase rapidly, the deeision was made in 2003 to onee again introduee hypoviruses. If biologieal eontrol ean be initiated on individual trees by eanker treatment, as seems to be indieated, the additional treatments may help save some trees and also help determine why viruses do not disperse to new eankers on trees that are untreated.

Table 2. Classification of cankers at West Salem, WI based on cultural morphology of *Cryphonectria parasitica* isolates removed from treated cankers, non-treated cankers on treated trees and non-treated cankers on non-treated trees from 1994-2000.

	Hypovirus-treated cankers		Non-treated cankers on treated trees		Non-treated cankers on non-treated trees	
Year Sampled	Hypovirulent	Virulent	Hypovirulent	Virulent	Hypovirulent	Virulent
1994	55%	45%	18%	82%	29%	71%
1995	55%	45%	22%	78%	0%	100%
1996	80%	20%	58%	42%	33%	67%
1997	82%	18%	42%	58%	9%	91%
1998	83%	17%	71%	29%	22%	78%
1999	81%	19%	71%	29%	28%	72%
2000	60%	40%	46%	54%	9%	91%

CONCLUSIONS

After its initial discovery, the prospects of utilizing hypoviruses to biologically control chestnut blight seemed reasonably straightforward and exploitable. The early successes achieved by treating infections on stems were in themselves remarkable. Unfortunately, hypovirus populations have not been perpetuated or disseminated adequately at sites where they have been artificially released, as has happened naturally at some locations. Admittedly, the phenomenon of hypovirulence, like most biological issues, is wrought with complexity. Whether we can duplicate artificially what has happened naturally remains a significant question. Clearly, major details of the epidemiology of chestnut blight and the infecting hypoviruses need to be unraveled. The successful transition of a virulent *C. parasitica* to one that is laden with debilitating hypoviruses is not regulated by a single factor. A summary of some of the components involved in the expression of hypovirulence that require further research include:

- an understanding of the contribution of chestnut genotype to the expression of hypovirulence;
- an appreciation of the role environmental factors play in contributing to the success or failure of hypovirulence;
- an evaluation of the pathogen population relative to its ability to cause disease, reproduce and acquire hypoviruses;
- an assessment of the influence specific hypovirus species have on *C. parasitica*;
- a consideration of potential vector relationships that might influence hypovirus dissemination; and,
- an evaluation of strategies for the deployment of hypoviruses.

We remain encouraged by the levels of recovery from blight that has occurred naturally at some locations. Over long biological time periods, hypovirulence may emerge on its own within the American chestnut's natural range. But, if man is to influence the process of biological control, a more complete understanding of the biology of the hypovirulence phenomenon is required.

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INTEGRATED USE OF RESISTANCE, HYPOVIRULENCE, AND FOREST MANAGEMENT TO CONTROL BLIGHT ON AMERICAN CHESTNUT

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Abstract: In the natural range of the species, the survival of most large American chestnut trees was associated with blight resistance, hypovirulence (reduced virulence) in the chestnut blight fungus, *Cryphonectria parasitica*, and favorable sites. Controlled intercrosses of these trees have resulted in progeny with acceptable levels of resistance, which have been used in further breeding. Some American chestnuts in a very large population (135,123 trees) derived from open pollinations of large survivors have shown promising levels of field blight resistance. In forest clearcuts and plantations, hypovirulence, associated with hypovirus infection in the blight fungus, develops naturally following blight epidemics. However, chestnut stems die due to: 1) the high blight susceptibility of American chestnut; 2) the rapid spread of vegetative compatibility-diverse, abundant, virulent inoculum; 3) the slow spread of hypovirulence; 4) high hardwood competition; and 5) low-temperature stress at high-altitude (> 2,500 feet) sites or drought. A long term (>20 years) and high level of blight control has been obtained on mesic, managed (control of competing hardwoods) sites, established with blight-resistant American chestnuts that were inoculated with a hypovirulent strain mixture. Cultural studies and nucleotide sequence analysis of two hypovirus regions (both >800 bp) indicated that blight control was associated with the spread of Italian *Cryphonectria hypovirus 1* (CHV1). Blight resistance may allow time for CHV1 to spread. Mesic shallow coves on lower altitude slopes are among the best sites to implement integrated blight management and restoration of American chestnut.

Key Words: American chestnut / Blight resistance / Hypovirulence / CHV1 / Forest management

INTRODUCTION

Following the chestnut blight pandemic, only a few timber-sized American chestnuts survived in the native range of the species, where it was once a dominant component of the former oak-chestnut and mixed mesophytic forest regions (Braun 1950). From the mid 1970s to the early 1980s, we investigated why these trees survived. The main focus was on tests for blight resistance, hypovirulence in the chestnut blight fungus, *Cryphonectria parasitica* (Murrill) Barr, and site factors. Several large trees were found, with the largest being greater than 40 inches in diameter at breast height (dbh). This tree grows at the base of the Northern Blue Ridge Mountains in Virginia. Understanding why these trees survived may aid in attempts to control the blight and restore the American chestnut.

EVALUATION OF BLIGHT RESISTANCE AND HYPOVIRULENCE IN LARGE, SURVIVING AMERICAN CHESTNUT TREES

The concept of disease resistance in plants, as viewed by American chestnut workers, both scientists and non-scientists, is often very restricted. Some believe a plant almost has to be immune to be labeled "resistant" to a given disease and that resistant plants never die from disease. Scientists working in the

field of disease resistance recognize that disease resistance is variable. For example, the grey scale shown in Fig. 1 shows how disease severity can vary from a very low level (represented by white) through increasing intensities of grey until the scale is black, representing high disease severity. These disease severity levels are then translated into disease resistance/susceptibility ratings, which, therefore, can vary from high disease resistance to high disease susceptibility. Some workers, such as disease-resistance scholar, J.E. Vanderplank (1982), have called the high disease resistance in Fig. 1 "full" or "complete resistance", and the high susceptibility "full" or "complete susceptibility". All in-between values were called "partial resistance" by Vanderplank. When chestnut stems of a different species or genotype were inoculated with a virulent strain of the chestnut blight fungus, disease severity ratings were continuously variable (Anagnostakis 1992; Bazzigher and Schmid 1962; Clapper 1952; Griffin et al. 1983; Hebard 1999), and thus resistance/susceptibility ratings can vary similarly, as represented in Fig. 1. The middle of the scale (see arrow) may be called partial resistance or moderate resistance (MR). The disease severity rating can be influenced by environmental factors, ontogenic factors, virulence of the pathogen, and the time interval over which the test is conducted (Bazziger and Schmid 1962; Griffin et al. 1986). Stressful environmental factors shown to be associated with more severe chestnut blight, such as early frosts (Berry 1951), growth in frost pockets or at high altitude (Headland et al.1976; Jones et al. 1980), drought (Goa and Shain 1995), and low light intensity (Uchida 1977), may increase disease severity (lower arrow by the scale). Factors favorable to the chestnut tree, such as optimal soil moisture (Goa and Shain 1995) over the growing season, a mesic site, and high intensity light (Uchida 1977), may lower disease severity (upper arrow by the scale) ratings (Fig.1). In the former oak-chestnut region, low temperatures at high altitudes and summer droughts are common events and can be stressful to chestnuts during blight resistance trials.

Several approaches were used by us to evaluate blight resistance in large, surviving American chestnut trees, including *in situ* inoculations of branches on the large, surviving trees with standard virulent strains of the blight fungus, inoculation of seedling progeny from large surviving trees growing at the same location, inoculation of grafts of the large survivors, and inoculation of excised stems of the survivors in the laboratory (Griffin et al.1983). Canker length and, in some tests, evaluation of cambium colonization and necrosis following canker dissection with a knife, were used to determine disease severity. In later trials, necrosis at the cambium was evaluated by bark-core sampling of the cankers. To evaluate hypovirulence of *C. parasitica* isolates recovered from the large, surviving trees, the procedure was similar except that blight-susceptible, clearcut, or understory American chestnut trees were inoculated with several pathogen isolates from each large, surviving tree. Site factors evaluated included elevation, competition from other hardwoods, slope, aspect, and soil characteristics. The results indicated that survival of most large trees was associated with low to moderate levels of blight resistance, a low frequency of hypovirulence in the blight fungus population on the tree, and favorable sites that were relatively free from competition. In 15 trials of 13 surviving trees, 73% showed significant evidence of blight resistance when compared to blight-susceptible reference trees, following *in situ* or seedling inoculations in the field with standard virulent strains (Griffin et al. 1983). For 317 blight fungus isolates recovered from 19 surviving trees, 28% were hypovirulent or intermediate hypovirulent in pathogenicity trials on forest American chestnut trees. Several trees exhibited no evidence of blight resistance, and a few trees were growing in competition with other trees. Mites recovered from large surviving trees were associated with virulent and hypovirulent *C. parasitica* and may be agents of hypovirulence spread on surviving trees (Wendt et al. 1983; Griffin et al.1984).

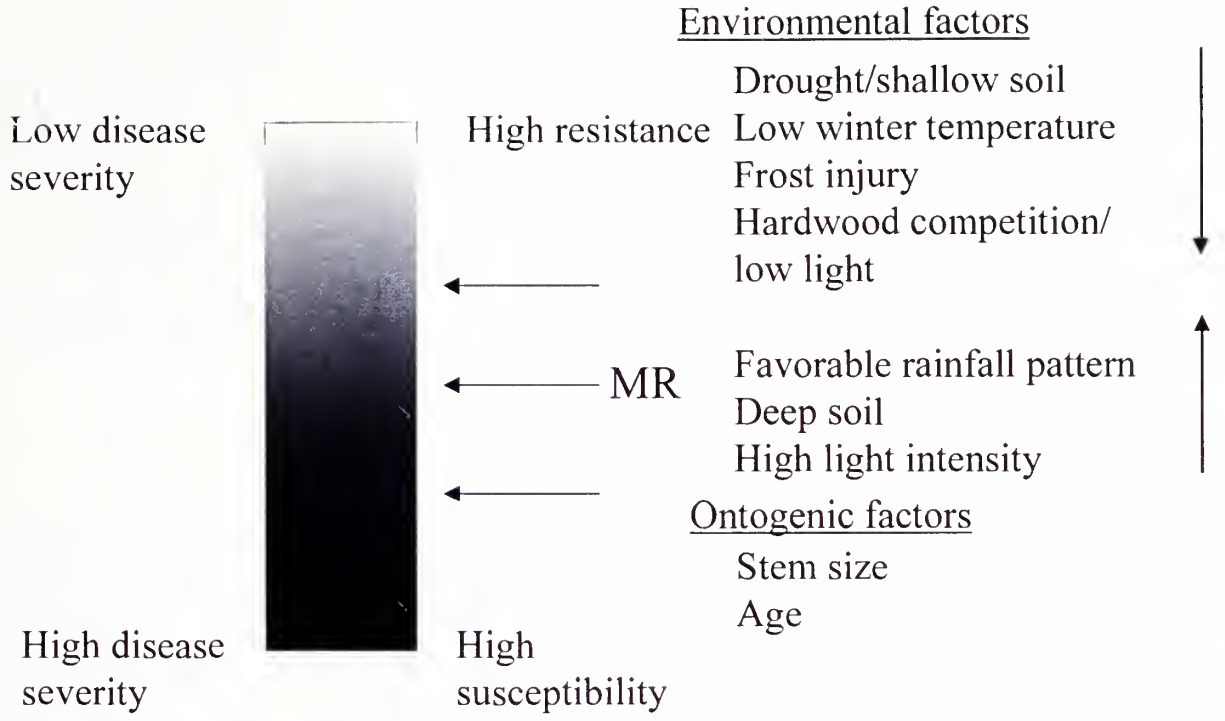

Figure 1. Concept of plant disease or chestnut blight resistance in relation to observed continuously variable disease severity, as represented by the grey scale bar. Low disease severity (whitish top) may be called high or complete disease resistance (*sensu* Vanderplank 1982), high disease severity (blackish bottom) called high or complete susceptibility, and intermediate disease severity (medium grey) may be called moderate resistance (MR) or partial resistance. For chestnut blight, in any given year or test, environmental and ontogenic factors can shift the disease severity rating and resistance rating up or down as shown (see arrows).

BREEDING AMERICAN CHESTNUT FOR BLIGHT RESISTANCE

Initially, four large, surviving trees (Fig. 2) were selected from this group of surviving trees to start an American chestnut blight-resistance breeding program. Controlled intercrosses were made among these trees. In this American Chestnut Cooperators' Foundation (ACCF) program, we are testing the hypothesis that additive blight resistance can be obtained through two or three cycles of selection. Vanderplank (1982) has documented that, in practice, gains of resistance through selection have been common and rapid in other host-pathogen systems. The evidence was that this additive resistance is mostly oligogenic. In our program, the progeny were grown at high elevation (> 2,500 feet), and after 7 years, these progeny were evaluated for blight resistance as described above. Two of the crosses, F x M and F x G, or the reciprocal cross, produced a high percentage of progeny that had acceptable disease severity ratings for further breeding. After one year of canker growth in these crosses, about one-half of the progeny trees had relative canker disease severity indexes (canker length x percent necrosis at the cambium) that indicated resistance when compared to results obtained for the blight-susceptible clone used in the progeny test. Also, this conclusion agrees with data obtained in the *in situ* tests mentioned above (Griffin et al. 1983), although strict comparisons to the latter cannot be made. The canker length and percent cambium necrosis components of the canker disease severity index for several progeny trees and their parents, obtained earlier, are shown in Fig. 2. The M (McDaniel) surviving tree, which had the

highest canker disease severity index of the four parent trees, combined well with the F (Floyd) surviving tree, as did the G (Gault) surviving tree. The WY (Weekly) surviving tree yielded low percentages of progeny that had acceptable disease severity ratings for further breeding. As these trials lasted one year at high altitude, they included the winter dormant season, which has been associated with low-temperature stress, canker development or expansion toward the vascular cambium, and possible breakdown of blight resistance at high elevations (see later sections). Using individual F_1 trees, F_2 progeny from the F x M and F x G crosses are now being grown. Backcrosses have been made to maintain some of the desirable traits of these locally-adapted parent trees. In addition, the progeny of several new intercrosses from trees showing high vigor and/or adaptation to different elevations are being gown as additional resistance sources.

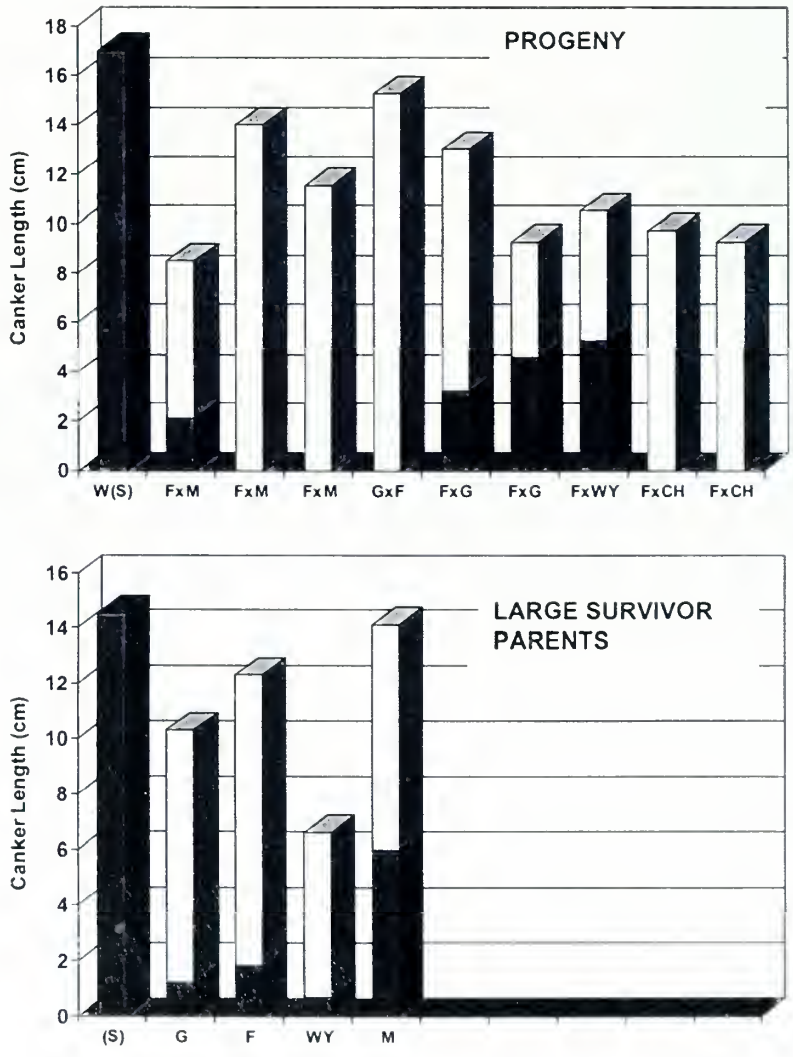

Figure 2. Canker lengths and percent of cambium areas beneath the cankers that are necrotic (shaded portion of bars) in 1-year duration blight resistance trials, with a standard virulent strain of *C. parasitica*, for (below) *in situ* inoculations on branches of the G, F, WY, and M large, surviving American chestnut parents, and (above) on main stems for 7-year-old intercross progeny of these surviving trees. The mean of seven susceptible reference trees (S) used is shown for the parents and the mean of one W(S) susceptible reference tree used for the progeny is shown. CH indicates Chinese chestnut reference parent.

A large population of American chestnuts with a potential for blight resistance are being grown by ACCF cooperators. These trees are progeny from open pollinations of a population of trees derived from large survivors and grown in the same breeding orchard. These breeding-orchard trees have exhibited low to moderate levels of blight resistance, and many are related to the parent trees used in the control pollinations. These trees likely share some of the same alleles that may be responsible for blight resistance and other desirable traits. The blight-resistant progeny of these open-pollinated trees should be good sources for increasing genetic diversity during later American chestnut restoration efforts. As of February, 2004, very large numbers of seedling transplants (93,643) and seed nuts (41,480) have been planted, and some have shown promising levels of field resistance to chestnut blight. The best of these trees, in terms of durable field blight resistance, can be incorporated into the controlled-cross breeding program described above. From these open pollinations, the National Park Service is now growing 4,500 seedling transplants and 462 trees from nuts.

INTEGRATION OF BLIGHT RESISTANCE AND HYPOVIRULENCE FOR CHESTNUT BLIGHT CONTROL

In 1980, John Elkins and Bruce Given (West Virginia Department of Agriculture), of the ACCF, worked with Tom Dierauf, of the Virginia Department of Forestry, to graft large, surviving American chestnuts on American chestnut rootstocks. These tree rootstocks were established 11 years earlier by Al Dietz (1978), founding officer of the ACCF, at the Lesesne State Forest, for the purpose of breeding a blight-resistant American chestnut by radiation breeding. The site in the Lesesne State Forest was 1,350 feet in elevation and mesic. Most of the trees in this radiation breeding program were dying from chestnut blight, but stump sprouts and the rootstocks survived. In 1982 and 1983, blight cankers on the American chestnut grafts were inoculated with a mixture of American and European dsRNA-infected, hypovirulent strains obtained from J.E. Elliston of the Connecticut Agricultural Experiment Station. Over the next two decades, these trees exhibited a high level of blight control, with blight cankers exhibiting a high degree of superficiality (Dierauf et al. 1997). By 1999, the largest tree had attained a height of 61 feet and a dbh of 15.7 inches (Griffin 2000). This same tree had a dbh of 19.0 inches in March, 2004 (Fig. 3). This blight control occurred in the presence of an abundant, virulent, ascospore inoculum generated by numerous perithecia in the thousands of cankers on the 5,000 American chestnut trees planted by Dietz in the Lesesne plantation. Inoculation trials with a standard virulent strain provided evidence for blight resistance in these grafts, and the virulent strain was later recovered from the superficial cankers resulting from the inoculations (Robbins and Griffin 1999). Some seedling American chestnut trees from the blight-resistant, large survivor intercrosses described above, also have exhibited a high to moderate level of blight control after 20 years when inoculated with a European hypovirulent strain mixture early or after several years of tree growth.

Extensive research indicated that dsRNA hypoviruses from the European hypovirulent strains had spread into 34-41% of isolates of the blight fungus recovered from the grafted trees at Lesesne, based on colony morphologies of over 800 *C. parasitica* isolates recovered from cankers (Griffin 1999; Robbins and Griffin 1999; Hogan and Griffin 2002 a and b). Colonies of European hypovirulent strains commonly have a predominantly white phenotype versus the orange-pigmented phenotype of dsRNA-free virulent, normal strains or many American hypovirulent strains. Assays of over 70 isolates of *C. parasitica* from the grafts, all with a predominantly orange-pigmented phenotype, indicated that most were free of hypovirus dsRNA. In white isolates, the European hypovirus, *Cryphonectria hypovirus 1* (CHV1, Hillman et al. 1995), had spread 642 cm from the hypovirulent–strain-inoculated zone and into a very large number (> 45) of vegetative compatibility types of the chestnut blight fungus (Hogan and Griffin 2002a). Blight resistance may have allowed time for CHV1 to spread. Vegetative incompatibility between different strains of the blight fungus was believed to be a major barrier to hypovirus transmission and biocontrol of chestnut blight in the United States, where a similar large number of vegetative

compatibility types of *C. parasitica* had been identified in research plots (Anagnostakis and Kranz 1987; Anagnostakis and Day 1979; Kuhlman and Bhattyacharyya 1984; Lui and Milgroom 1996). Within a canker, however, incomplete movement of CHV1 within a vegetative compatibility type was found for the natural cankers on the grafts (Hogan and Griffin 2002b).

A few predominantly orange-pigmented isolates from the grafts had high dsRNA content, and high dsRNA is characteristic of European hypovirulent strains (Dodds 1980). The European hypovirulent strains inoculated on the grafts were of French and Italian origin. The French strain inoculated on the

Figure 3. Integrated blight control on American chestnut with resistance and hypovirulence. Photo taken in March, 2004 of a 19.0-inch dbh stem (left) and an 18.5-inch dbh stem (right) of a two-stem American chestnut tree grafted in 1980 from a large survivor and later inoculated with a mixture of hypovirulent strains of the blight fungus. Arrow points to a highly superficial (nonkilling) canker. This high level of blight control is typical throughout this tree, which is over 60 feet tall. Bars on scale to right are 1 foot long.

grafts had a predominantly orange-pigmented phenotype, but was derived from a predominantly white hypovirulent strain (Elliston 1985). The inoculated Italian hypovirulent strains had predominantly white phenotypes. Single-spore analysis of the white isolates from the grafts suggested that hypovirus from the Italian inoculated strains had spread on the grafts (Hogan and Griffin 2002a). Colony morphologies of hypovirulent strains are variable in subculture, however. Therefore, nucleotide sequence identification was used to determine the identity of CHV1 in the white isolates and to determine the identity of CHV1 in the predominantly pigmented isolates as French or Italian.

To identify CHV1 as French or Italian, hypovirus dsRNA was extracted from predominantly white and predominantly pigmented *C. parasitica* isolates recovered from cankers on the grafts. cDNAs were then made by reverse transcriptase-polymerase chain reaction (RT-PCR) for two hypovirus regions: (1) an 844-bp region in the helicase domain of open reading frame B (ORF B), and (2) an 894-bp region that included part of the 5′ non-coding region and part of p29 of ORF A. Nucleotide sequence analysis indicated that all pigmented and white *C. parasitica* hypovirus isolates from the grafted trees and Italian inoculated strains had high identities to each other and high identities (98.7-99.9%) to the Italian reference hypovirus, CHV1-Euro7 (Griffin et al. 2004). Identities to French reference hypovirus CHV1-EP713 were low (<89.8%). Thus, no evidence was found for the presence of French hypovirus, except for the hypovirus in the predominantly orange-pigmented French hypovirulent strain inoculated on the trees.

FOREST MANAGEMENT IN CLEARCUTS AND PLANATIONS FOR INTEGRATED CHESTNUT BLIGHT CONTROL AND RESTORATION OF AMERICAN CHESTNUT

American chestnut presently may be found sparsely to frequently as an understory tree throughout the native range of the species in both the former oak-chestnut and mixed mesophytic forest regions (Braun 1950). Xeric and intermediate sites may have very high population densities of understory American chestnuts, especially at elevations of about 3,000 feet or higher in the Mid-Atlantic area of the former oak-chestnut region. Highly mesic sites may have little or no understory American chestnut survival (Griffin 1992a). Chestnut blight is endemic in these understory trees with about 15-20% blight incidence. When these areas are clearcut, American chestnut grows as rapidly as any hardwood in the clearcut (Smith 1977). This rapid growth is followed by a chestnut blight epidemic over a 10-year period when 90-100% of the chestnut trees are blighted. A great abundance of virulent ascospore inoculum develops from perithecia on hundreds of American chestnut stems in these clearcuts. Similar epidemics occur in blight-susceptible American chestnut plantations. Near the end of the epidemic, some trees exhibit superficial cankers which are associated with hypovirulent strains, some possibly originating from cankers in the understory American chestnuts (Griffin et al. 1983; Griffin et al. 1984). On xeric and intermediate sites, following stem death from blight, numerous stump sprouts develop, some of which are browsed by deer. On mesic sites, with high competition from hardwoods, few stump sprouts develop, almost all of which are browsed by deer. On these sites with great tree-growth potential, American chestnut rootstocks are completely lost. Survival of American chestnut over all sites was inversely related to basal area of competing hardwoods (Griffin et al. 1991). Light intensities were very low on sprouts at the base of the stump on the mesic sites (Griffin 1992b).

Forest management involving removal of competing hardwoods resulted in the development of superficial cankers that were associated with dsRNA-infected hypovirulent strains of the chestnut blight fungus (Griffin et al. 1991; Griffin et al. 1993). This management practice increased stem size, promoted mast production, and maintained the survival of chestnut stems for several years beyond that found in check plots having no removal of competing hardwoods. The greatest blight control was found on a mesic site that was clearcut and a mesic plantation site (Griffin et al. 1991). However, at all locations blight control

eventually broke down. Blight control also broke down in clearcuts where hypovirulent strains were artificially introduced. Research indicated that this breakdown of biocontrol was associated with the following: 1) the high blight susceptibility (quick kill) of forest American chestnuts; 2a) the secondary colonization of superficial (hypovirulent) cankers by virulent strains in diverse vegetative compatibility types that were generated in the clearcut or plantation; 2b) the development of new killing cankers elsewhere on the chestnut stem by virulent strains; 3) the slow spread of hypovirulence; and 4) breakdown at high altitude of superficial (hypovirulent) cankers over winter (Griffin et al 1993; Griffin and Griffin 1995). In the absence of hardwood management, even in unmanaged plantations, factor 5) is high hardwood competition and the associated reduced light. All lead or contribute to chestnut stem death. Using artificially introduced hypovirulent strains in forest situations, others have also found blight control to be either unsuccessful (Liu et al. 2002) or partial (Anagnostakis 2001). Further, introduced European hypoviruses (CHV1) did not persist at sites where they were introduced (Liu et al. 2002; Peever et al. 1997).

In our study, the clearcut and plantation blight-susceptible trees grew at altitudes ranging from 2,000 to 3,500 feet. In contrast, some blight-susceptible American chestnuts naturally infected with hypovirulent strains at low altitudes (< 1,000 feet elevation) have exhibited durable blight control, even in the absence of blight resistance (Griffin et al. 1983; Griffin and Griffin 1995). When pure cultures of hypovirulent strains and bark plugs from a low altitude tree were inoculated into American chestnut trees at high altitude (3,500 feet in elevation), superficial cankers were produced during the growing season. However, the hypovirulent strains colonized the cambium over the winter, causing a breakdown in the canker superficiality rating and biocontrol (Griffin and Griffin 1995). Tests indicated that the hypovirus in the hypovirulent strains survived the winter. These trees died as the cambium was completely killed. Conversely, the original low-altitude American chestnut tree (hypovirulence source) still exhibited stable blight control as of March, 2004 (unpublished).

As indicated above, physiological stress in chestnut species may occur at high altitudes, from early frosts, and in frost pockets. At these sites, blight severity can be very high even on the highly blight-resistant Chinese chestnut and some stems can be killed (Berry 1951; Headland et al 1976; Jones et al. 1980). Other studies have indicated that American chestnut at high altitude sites are under physiological stress. This is indicated by greatly increased electrolyte leakage from bark tissues collected from high altitude (3,900 feet) versus low altitude (530 feet) sites (Griffin 2000). The above findings suggest that the blight control breakdown at high-altitude clearcuts described above may be related in part to physiological stress. Some large-surviving American chestnut trees have been found at elevations higher than 4,000 feet, and these trees, adapted to blight at these elevations, may be useful for breeding and integrated blight control at higher elevations. High elevation sites (>2,500 feet elevation) may account for the bulk of the surviving population of understory American chestnuts in Virginia

Physiological stress on American chestnuts may also occur during the summer drought periods commonly encountered in the former oak-chestnut forest region (Braun 1950). Gao and Shain (1995) found drought stress may contribute to blight susceptibility. Additionally, Anagnostakis (2001) found that in Connecticut forest plots, where hypovirulence was artificially introduced, American chestnut trees with many cankers often died in the summer following drought the previous year. As indicated above, biocontrol associated with natural hypovirulence was less on managed, xeric clearcut sites than on managed, mesic clearcut sites (Griffin et al. 1991). Xeric slope sites frequently have small American chestnut stumps and very high population densities of understory American chestnuts that are associated with a less dense canopy. This can lead to the false conclusion that they are the best chestnut restoration sites. In contrast, mesic shallow cove sites on slopes had large American chestnut stumps (up to 3-4 feet in diameter) with moderate numbers of understory American chestnuts (Griffin 1992a). Deep cove sites had medium-sized stumps and little or no understory American chestnuts. Mesic shallow cove sites on slopes have great potential for chestnut growth, integrated blight control with resistance, hypovirulence,

and forest management, chestnut restoration, and natural regeneration of American chestnut. Often they have deep, fertile soils. Large-surviving American chestnuts at high altitude (>2,500 feet) may be useful for breeding and integrated control at higher elevation, mesic sites.

CONCLUSIONS

Research indicated that the survival of most large surviving American chestnut trees was associated with resistance, hypovirulence, and favorable sites. Our ACCF breeding program utilizes controlled intercrosses of these trees, which has resulted in progeny with acceptable levels of resistance. These progeny trees have been used in further breeding. This may result in trees with additive blight resistance. Some American chestnuts in a very large population (135,123 trees), derived from open pollinations of large survivors, have shown promising levels of field blight resistance. This large population may serve as a source of genetic diversity in future restoration efforts. The high blight susceptibility of forest American chestnuts, along with the abundance of a vc-diverse, virulent inoculum of *C. parasitica* has severely limited blight control and the use of hypovirulence in forest clearcuts and plantations. High altitude, low temperature stress, drought stress, and hardwood competition are additional factors that inhibit blight control and the use of hypovirulence. However, a long term (> 20 years) and high level of blight control has been obtained on mesic, hardwood-managed sites. These sites were established with blight-resistant American chestnut trees that were inoculated with hypovirulent strains of *C. parasitica*. Nucleotide sequence analysis indicated that blight control was associated with the spread of Italian CHV1. Some blight resistance may be needed in American chestnut to allow time for hypoviruses to spread. Site selection and removal of competing hardwoods may be critical forest management practices needed for blight control. Mesic shallow coves on lower altitude slopes are among the best sites to implement integrated use of resistance, hypovirulence, and forest management for blight control and restoration of American chestnut. High-altitude (>2,500 feet), large-surviving American chestnuts may be useful for breeding and integrated control at higher elevation, mesic sites.

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GENETIC STRUCTURE OF AMERICAN CHESTNUT POPULATIONS BASED ON NEUTRAL DNA MARKERS

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Abstract: Microsatellite and RAPD markers suggest that American chestnut exists as a highly variable species, even at the margins of its natural range, with a large proportion of its genetic variability occurring within populations (~95%). A statistically significant proportion also exists among populations. Although genetic differentiation among populations has taken place, no disjunct regional pattern of variation exists. A cline in allele frequencies and number of rare alleles occurs along the Appalachian axis, with the highest levels of gene diversity and the greatest numbers of rare alleles being found in southwestern populations. Population pairwise estimates of genetic distance are significantly associated with the geographic distance between populations. Geographically proximate populations are slightly more genetically similar than geographically distant populations. Genetic variability in American chestnut follows a pattern consistent with the hypothesis of a single metapopulation in which genetic drift plays a major evolutionary role. Results of this study are based on neutral genetic loci and do not necessarily reflect genetic differentiation for adaptive genes or gene complexes. Therefore, in order to assure that most of the variation at these genes is also captured in conservation and breeding endeavors, sampling should focus on collecting a fairly large number of individuals from each of several geographic areas.

Keywords: *Castanea dentata* / SSR / RAPD / genotypic diversity / haplotype diversity

INTRODUCTION

The American chestnut (*Castanea dentata* Borkh.) was once one of the most important timber and nut-producing tree species in eastern North America (U.S. Census Bureau 1908). Its native range extended from southern Maine and Ontario in the north to Georgia, Alabama and Mississippi in the south (Sargent 1905). The species now exists primarily as stump sprouts across this entire range, the victim of a devastating canker disease. The disease, chestnut blight, is caused by an exotic fungal pathogen now known systematically as *Cryphonectria parasitica* (Barr 1979). After more than half a century of blight, numerous living stems of American chestnut still exist in the understory of upland forests in the mid-Appalachians (Stephenson et al. 1991). Prolific stump sprouting has enabled American chestnut to persist, but as sexual reproduction is infrequent, its gene pool will likely face serious erosion when old root systems fail to produce sprouts and perish.

Because resistance to *C. parasitica* is low or lacking in American chestnut, Burnham (1981) proposed the use of a classical backcross breeding program to develop blight resistant timber-type trees. Adopting this methodology as their charter, the non-profit philanthropic organization The American Chestnut Foundation (TACF) has since developed a vigorous backcross breeding program designed to introgress the resistance of Chinese chestnut (*C. mollissima* Blume) into American chestnut (Hebard 1994; Kubisiak et al. 1997). TACF's initial efforts focused on American chestnut trees in southwest Virginia, but the goal is to restore the species throughout its entire native range. Thus, information regarding the amount and distribution of molecular genetic variation in American chestnut might help to better determine the number of breeding locations that will be needed across the species range.

Previously, little was known about how genetic variability is distributed across the landscape that comprises the natural range of this species. In an exploratory examination of genetic variability for American chestnut, Huang et al. (1998) obtained results with allozyme and random amplified polymorphic DNA (RAPD) markers that suggest as many as four regional metapopulations might exist. However, hierarchical AMOVA was not performed to quantify this putative regional component, nor were statistical tests employed to test for significant differences. Since that research was completed, the magnitude, significance, and patterns of regional structure have been the subjects of much discussion and debate (F.V. Hebard, P. Sisco, and G. Miller personal communication). Given the importance of regional structure in regards to breeding blight resistant regionally adapted American chestnut, we felt compelled to embark on a more thorough examination of genetic variation in American chestnut using microsatellite and RAPD markers.

Here we report results obtained from an analysis of genetic structure for populations of American chestnut occurring over a significant portion of its natural range. We assayed six microsatellite and 19 RAPD markers and based our analysis on allele and haplotype frequency variation observed for these neutral loci. Our objective for this research was to secure a more detailed and complete understanding of population structure for American chestnut. In the following sections we describe genetic differentiation patterns observed within and among populations and report estimates of diversity parameters associated with microsatellite and RAPD loci segregating in American chestnut. Finally, we compare our results to patterns of variability previously reported for neutral markers in American chestnut as well as in other tree species.

MATERIALS AND METHODS

Population sampling and DNA extraction

A rangewide sampling of expanded leaves or dormant buds of American chestnut were collected at 22 sites across its natural range (refer to Figure 1). Most of the samples were collected from sites in State or National Forests, but a few sites were located on private land holdings. Each sample was assigned a unique ID and sent to the Southern Institute of Forest Genetics in Saucier, Mississippi for DNA extraction and analysis. Total nucleic acids were isolated from tree tissues as described in Kubisiak et al. (1997).

Species evaluation

A panel of DNAs consisting of eight American chestnut (one from each of eight different sites sampled for this study), six Chinese chestnut (trees from USDA import #'s 70315, 104061, 78626, 104014, 104015, and 104016), seven Henry chinkapin (*C. henryi* Rehder & Wils.) (trees from USDA import # 104058, the Nanjing Botanical Garden, Nanjing, Peoples Republic of China (PRC), and the Wuhan Institute of Botany, Wuhan, PRC), four Seguin chestnut (*C. seguinii* Dode) (trees from USDA import # 70317), seven European chestnut (*C. sativa* Mill.) (including trees from the Caucasus Mountains of southern Russia, Bursa, Turkey, and the Black Forest in Germany), and eight Alleghany chinkapin (*C. pumila* Mill.) (Harrison County, Mississippi) were amplified using the polymerase chain reaction (PCR) and a chloroplast-specific primer pair (a, b) as described in Taberlet et al. (1991).

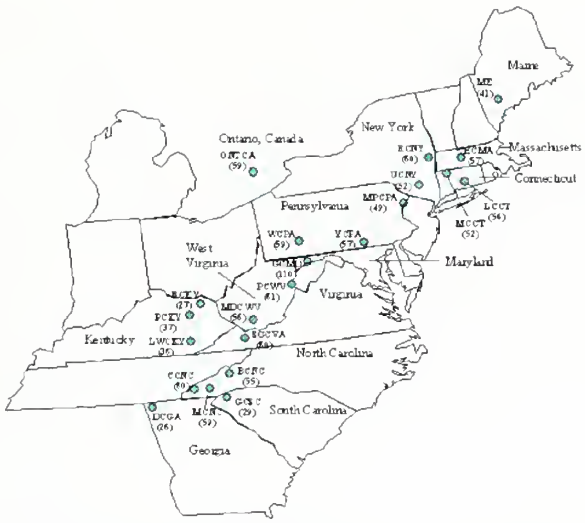

Figure 1. Map of the geographic origin of the 22 *Castanea dentata* Borkh. populations sampled in this investigation. The number in parentheses refers to the number of trees sampled at each location.

Microsatellite PCR amplification and detection

Primer sequences and PCR conditions for microsatellite loci developed in European chestnut (*C. sativa*) were obtained from the literature (Marinoni et al. 2003). Primer sequences for microsatellite loci developed in white oak (*Quercus alba* L.) were obtained from A. David and D. Wagner at the University of Kentucky. For each microsatellite, the forward primer was 5'-end labeled with one of three fluorescent dyes to facilitate detection using the Applied Biosystems 3100 Genetic Analyzer and the GENESCAN® version 3.7 fragment analysis software (Applied Biosystems, Inc. Foster City, CA). Microsatellites were PCR amplified and the products post-PCR multiplexed by color and size whenever possible. Allele sizes were determined by including the GENESCAN®-500[TAMRA] internal size standard in each sample lane. The data were scored using GENOTYPER® version 3.7 (Applied Biosystems, Inc. Foster City, CA).

RAPD PCR amplification and detection

RAPD amplification and detection was based on the protocols reported in Kubisiak et al. (1997). RAPD fragments were identified by the manufacturer primer code corresponding to the primer responsible for their amplification, followed by a subscript four digit number indicating the approximate fragment size in base pairs. Markers were chosen based on the intensity of amplification (only intensely amplified bands were scored) and the absence of co-migrating DNA fragments. All markers were found to conform to Mendelian expectations based on their inheritance in at least one of four different interspecific chestnut pedigrees.

Data analysis

A search for common/redundant multi-locus genotypes and haplotypes was performed (Excoffier and Slatkin 1995). Populations were tested for Hardy-Weinberg proportions using both χ^2 and G^2 tests (Weir 1990). Allele frequencies for each population were computed and estimates obtained for effective number of alleles per locus (A_e), Nei's (1972) measures of gene diversity (h), Nei's (1978) unbiased

measure of genetic distance (D), Michalakis and Excoffier's (1996) genetic differentiation measure (Φ_{ST}) for the microsatellite loci, and Nei's (1987) genetic differentiation measure (G_{ST}) for RAPD loci using the software program ARLEQUIN version 2.001 (Schneider et al. 2000) and POPGENE version 1.31 (Yeh et al. 1997). In addition, χ^2 and G^2 tests were calculated to test homogeneity of allele frequencies among populations. For microsatellite analysis in ARLEQUIN, alleles were coded assuming a step-wise mutation model. Associations between allele frequency and latitude or longitude were first studied using the PROC STEPWISE procedure in SAS version 8.01(SAS, 1999). A variable was only added to the model if its F-statistic was significant at the 5% level. Once added, any variable that did not have a F-value significant at the 5% level was deleted from the model. Associations between the observed number of alleles per locus, number of rare alleles per locus (rare alleles are those with frequencies less than 0.05 computed across all populations), effective number of alleles per locus, gene diversity and latitude or longitude were also studied. In order to further investigate any apparent clinal trends, a composite dependent variable (CDV) was computed that combined both latitude and longitude. First, a reference line was drawn between the southwestern most and northeastern most populations. Then, perpendicular lines were drawn that connected the various populations to this line. Distances (converted into kilometers) along the reference line to the population perpendiculars were used as values for CDV. Genetic distance (D) and among population differentiation were calculated for each pair of populations and associations with geographic distance were investigated using the PROC REG procedure in SAS. Genetic associations existing among populations were first studied using unweighted pair-group mean analysis (UPGMA) based on the matrix of Nei's genetic distance, and then by principal components analysis (PCA) conducted on allele frequency data using the PROC PRINCOMP procedure in SAS.

RESULTS

Putative species identification

Primers that amplified the intergenic spacer region between *trn*T (UGU) and the *trn*L (UAA) 5' exon of the chloroplast genome (primers a and b: 5'-CATTACAAATGCGATGCTCT-3' and 5'-TCTACCGATTTCGCCATATC-3', respectively; Taberlet et al. 1991) were found to uniquely differentiate American chestnut chloroplast DNA from all other *Castanea* (chestnut and chinkapin) species. Based on DNA sequence data (data courtesy F. Dane and P. Lang of Auburn University) this primer pair was found to amplify a band 857 base pairs (bp) in length in American chestnut, and bands ranging from 942 to 945 bp in all other *Castanea* species including the native chinkapin (both *C. pumila* var. *alleghaniensis* and *C. pumila* var. *ozarkensis*). Much of the size difference observed between American chestnut and the other *Castanea* species was due to two unique deletions (one 12 bp and the other 75 bp in length) contained within this region of the American chestnut chloroplast genome. A larger sampling of native chinkapin (specifically *C. pumila*; var. *alleghaniensis* - 48 trees) has yet to show the presence of these large deletions.

Based on the phenotype observed for this marker, of the 1158 chestnut trees sampled for this study 165 (14.2%) were eliminated from further analysis as they did not have the smaller chloroplast band characteristic of American chestnut. These 165 trees were collected from nine different sample sites. Four of the nine sites had very few suspect trees. One site had to be completely eliminated from the study as all trees sampled were found to be suspect. Four sites had to be pooled with the most geographically proximate site in the same state as a large number of suspect trees were found. In total, as many as 993 trees from 18 different sample sites were available for analysis of genetic variation in American chestnut.

Microsatellite-based genetic differentiation

Data describing the microsatellite loci used in our analyses are presented in Table 1. Considerable variation was displayed by the 6 loci. Five of the six loci had very little missing data and were thus used to search for common or redundant multilocus genotypes (MLGs) and haplotypes. Based on these five loci, only five redundant MLGs were observed. Each redundant MLG was only found to occur twice. Based on the same five loci, 114 of 1603 estimated haplotypes were found to occur more than once either within or across populations, but there was no apparent geographic trend to their distribution.

Table 1. Microsatellite and RAPD primer sequence, repeat type, allele size, and number of unique alleles identified in samples collected from 18 populations of *Castanea dentata* Borkh. located throughout the species natural range in eastern North America.

Locus	Primer Sequence 5'-3'	Repeat type	Allele size (bp)	Number of unique alleles
Microsatellites				
*Cs*CAT01[a]	F[b]:AGAATGCCCACTTTTGCA R:CTCCCTTATGGTCTCG	$(AC)_nAT(AC)_n$	167-211	31
*Cs*CAT14	F:GAGGTTGTTGTTCATCATTAC R:ATCTCAAGTCAAAAGGTGTC	$(AC)_n$	121-151	15
*Cs*CAT15	F:TCTGCGACCTCGAAACCGA R:CTAGGGTTTTCATTTCTAG	$(AG)_n$	115-141	15
*Qa*CA022	F:AACAATAGGAGTTGGTTTGAG R:GTTAGGGTTTGGAAAATAGGA	$(AC)_n$	160-188	13
*Qa*GA068	F:GCTTTTCTTTCCAGGGCTAC R:GTGGGACAGTGAGGCAGAG	$(AG)_n$	156-192	17
*Qa*GA209	F:CAAGCAGTATTGTTTTATCTC R:GTTGCCCCTGTGAACTAC	$(AG)_n$	227-265	15
RAPDs				
106	CGTCTGCCCG	NA	500	2
			525	2
			650	2
			700	2
			800	2
184	CAAACGGCAC	NA	450	2
			1150	2
			1800	2
213	CAGCGAACTA	NA	900	2
		NA	1000	2
225	CGACTCACAG	NA	800	2
			1450	2
237	CGACCAGAGC	NA	825	2
			1000	2
			1250	2
423	GGGTCTCGAA	NA	600	2
			875	2
500	TTGCGTCATG	NA	775	2
514	CGGTTAGACG	NA	575	2

[a]Locus names beginning with *Cs* were derived from *Castanea sativa* (Marinoni et al. 2003) and those beginning with *Qa* were derived from *Quercus alba* (sequences courtesy of A. David and D. Wagner). RAPD primer sequences were obtained from J. Hobbs at the University of British Columbia, BC, Canada.
[b]F=forward primer, and R=reverse primer

The expected genotype frequencies at all loci, and in all populations, conformed to Hardy-Weinberg expectations, except for locus *Qa*GA209 in population PCKY that showed a significant excess of homozygotes. Frequencies for alleles at greater than 10% frequency over all populations, plus those found to be significantly associated with latitude and/or longitude, are displayed by population in Table 2.

All six single-locus contingency χ^2 analyses as well as G^2 tests for homogeneity of allele frequency across populations indicated significant ($p<0.05$) departures from homogeneity.

Table 2. Microsatellite allele frequencies estimated for 18 populations of *Castanea dentata* Borkh. located throughout the species natural range in eastern North America. Only those alleles at frequencies greater than 0.1 over all populations and those alleles significantly associated with latitude or longitude (identified in italic and ***bold-italic***, respectively) are presented.

Locus and				west								east					
Allele (bp)	CCNC	BCNC	GCSC	PCKY	RCKY	SGCVA	ONTCA	PCWV	GCMD	WCPA	YCPA	MPCPA	UCNY	LCCT	RCNY	HCMA	MCCT	ME
CsCAT01																		
182	*0.0303*	*0.0098*	*0.0517*	*0.0096*	*0.0000*	*0.0424*	*0.0000*	*0.0067*	*0.0000*	*0.0000*	*0.0000*	*0.0000*	*0.0000*	*0.0357*	*0.0000*	*0.0179*	*0.0200*	*0.0000*
186	***0.0455***	***0.0098***	***0.0172***	***0.1058***	***0.1154***	***0.0508***	***0.0172***	***0.0467***	***0.0000***	***0.0179***	***0.0000***	***0.0294***	***0.0000***	***0.0000***	***0.0000***	***0.0000***	***0.0000***	***0.0000***
187	***0.0000***	***0.0196***	***0.0000***	***0.0000***	***0.0000***	***0.0339***	***0.0603***	***0.0267***	***0.0693***	***0.0714***	***0.0357***	***0.0980***	***0.1489***	***0.0536***	***0.1379***	***0.2232***	***0.0900***	***0.1125***
190	0.0455	0.1765	0.1034	0.0577	0.0769	0.0593	0.0690	0.0867	0.1832	0.0714	0.0804	0.1275	0.1702	0.2143	0.3448	0.0357	0.1000	0.1500
191	***0.0000***	***0.0000***	***0.0517***	***0.0096***	***0.0000***	***0.0000***	***0.0603***	***0.0133***	***0.0990***	***0.0089***	***0.0179***	***0.0196***	***0.0957***	***0.1429***	***0.1034***	***0.0446***	***0.0500***	***0.0625***
192	***0.1439***	***0.1569***	***0.0862***	***0.1346***	***0.0769***	***0.0593***	***0.0517***	***0.0600***	***0.0545***	***0.0089***	***0.0089***	***0.0000***	***0.1064***	***0.0089***	***0.0690***	***0.0089***	***0.0000***	***0.0125***
194	0.0985	0.1275	0.0690	0.0769	0.2115	0.0763	0.1638	0.1467	0.0693	0.1607	0.3036	0.0980	0.0957	0.2679	0.0776	0.0625	0.0700	0.0875
196	0.3409	0.1373	0.2241	0.2692	0.1538	0.1864	0.1897	0.1933	0.3218	0.3929	0.3929	0.2647	0.2872	0.2411	0.1466	0.1429	0.0800	0.1500
200	***0.0682***	***0.0588***	***0.0345***	***0.0481***	***0.0962***	***0.1102***	***0.0862***	***0.0400***	***0.0050***	***0.0000***	***0.0000***	***0.0490***	***0.0000***	***0.0089***	***0.0259***	***0.0089***	***0.0000***	***0.0375***
202	*0.0076*	*0.0098*	*0.0690*	*0.0000*	*0.0000*	*0.0000*	*0.0000*	*0.0067*	*0.0050*	*0.0000*	*0.0268*	*0.0000*	*0.0000*	*0.0000*	*0.0000*	*0.0000*	*0.0000*	*0.0000*
CsCAT14																		
129	0.1045	0.1373	0.1552	0.1458	0.3077	0.1780	0.2500	0.2403	0.2892	0.2632	0.3661	0.1389	0.3043	0.1455	0.2500	0.1792	0.4314	0.1750
133	0.3284	0.6078	0.4483	0.4792	0.4808	0.5000	0.4310	0.4091	0.4069	0.3158	0.3661	0.3796	0.2147	0.6000	0.5690	0.4717	0.3725	0.5125
143	*0.0000*	*0.0000*	*0.0000*	*0.0000*	*0.0000*	*0.0000*	*0.0259*	*0.0000*	*0.0196*	*0.0000*	*0.0000*	*0.0000*	*0.0213*	*0.0000*	*0.0086*	*0.0566*	*0.0000*	*0.0125*
145	***0.0075***	***0.0098***	***0.0000***	***0.0104***	***0.0000***	***0.0000***	***0.0000***	***0.0000***	***0.0000***	***0.0000***	***0.0000***	***0.0000***	***0.0000***	***0.0000***	***0.0000***	***0.0000***	***0.0000***	***0.0000***
CsCAT15																		
115	*0.0104*	*0.0185*	*0.0000*	*0.0000*	*0.0000*	*0.0000*	*0.0000*	*0.0000*	*0.0000*	*0.0000*	*0.0000*	*0.0000*	*0.0000*	*0.0000*	*0.0000*	*0.0000*	*0.0000*	*0.0000*
117	*0.0104*	*0.0926*	*0.1250*	*0.0135*	*0.1538*	*0.0833*	*0.0000*	*0.1346*	*0.0309*	*0.0122*	*0.0167*	*0.0132*	*0.0000*	*0.0000*	*0.0000*	*0.0000*	*0.0000*	*0.0357*
121	*0.0104*	*0.1111*	*0.0750*	*0.1622*	*0.1538*	*0.0833*	*0.1129*	*0.0385*	*0.2037*	*0.2805*	*0.0333*	*0.1316*	*0.2273*	*0.1383*	*0.3021*	*0.1410*	*0.0500*	*0.1786*
127	*0.0521*	*0.0556*	*0.0500*	*0.0270*	*0.0769*	*0.0833*	*0.0000*	*0.0385*	*0.0000*	*0.0000*	*0.0000*	*0.0000*	*0.0758*	*0.0000*	*0.0000*	*0.0000*	*0.0128*	*0.0000*	*0.0000*
133	0.2708	0.1111	0.1250	0.1486	0.1154	0.1000	0.2097	0.2500	0.1852	0.1951	0.1333	0.2368	0.1818	0.1064	0.1042	0.2436	0.2000	0.2500
137	*0.0521*	*0.1481*	*0.0000*	*0.0405*	*0.0385*	*0.0500*	*0.1935*	*0.1250*	*0.2037*	*0.0854*	*0.2000*	*0.1316*	*0.1212*	*0.2766*	*0.3646*	*0.3333*	*0.1000*	*0.2500*
139	***0.1042***	***0.0370***	***0.0000***	***0.1081***	***0.0769***	***0.1000***	***0.0806***	***0.1154***	***0.0370***	***0.0854***	***0.0667***	***0.1184***	***0.0152***	***0.0319***	***0.0000***	***0.0128***	***0.0125***	***0.0357***
QaCA022																		
170	0.4141	0.6058	0.4655	0.2885	0.2500	0.2500	0.2586	0.2123	0.5727	0.5603	0.3482	0.3627	0.4688	0.4286	0.4741	0.2411	0.2000	0.2683
172	***0.1797***	***0.2308***	***0.2414***	***0.2596***	***0.1538***	***0.2679***	***0.1810***	***0.2308***	***0.1591***	***0.1207***	***0.1964***	***0.3824***	***0.3125***	***0.3661***	***0.3362***	***0.3036***	***0.5100***	***0.3049***
174	*0.0312*	*0.0192*	*0.0345*	*0.0192*	*0.0385*	*0.0446*	*0.0086*	*0.0137*	*0.0000*	*0.0086*	*0.0089*	*0.0000*	*0.0000*	*0.0000*	*0.0000*	*0.0000*	*0.0000*	*0.0122*
182	0.0234	0.0288	0.0172	0.0769	0.0962	0.2232	0.2672	0.1438	0.0955	0.0517	0.1786	0.0686	0.0312	0.1071	0.0862	0.0893	0.1000	0.1463
QaGA068																		
164	*0.0758*	*0.0000*	*0.1379*	*0.0943*	*0.0400*	*0.0000*	*0.0000*	*0.0063*	*0.0140*	*0.0000*	*0.0000*	*0.0000*	*0.0000*	*0.0000*	*0.0000*	*0.0000*	*0.0000*	*0.0122*
166	0.2955	0.1250	0.0517	0.0943	0.0400	0.0455	0.0259	0.0649	0.0374	0.0882	0.0818	0.0283	0.1979	0.2455	0.1810	0.1071	0.0769	0.0732
168	0.1515	0.0865	0.1379	0.2642	0.4400	0.0273	0.0345	0.2857	0.1869	0.1176	0.0545	0.0755	0.2083	0.1727	0.3190	0.3214	0.1250	0.1220
170	0.2273	0.2019	0.1034	0.2358	0.1800	0.2455	0.1207	0.1753	0.1589	0.0098	0.2091	0.3774	0.1771	0.0818	0.1724	0.2946	0.0673	0.3537
172	0.0530	0.1250	0.2069	0.0943	0.0200	0.3455	0.2931	0.3312	0.3224	0.3824	0.3909	0.2547	0.1458	0.0909	0.0603	0.1339	0.0577	0.2073
174	0.0909	0.0962	0.0517	0.0566	0.1600	0.1182	0.0086	0.0325	0.0935	0.0490	0.1636	0.2453	0.0938	0.2636	0.0690	0.0714	0.2115	0.0732
180	***0.0076***	***0.0096***	***0.0345***	***0.0943***	***0.0400***	***0.0273***	***0.0086***	***0.0260***	***0.0000***	***0.0098***	***0.0000***	***0.0000***	***0.0000***	***0.0000***	***0.0000***	***0.0179***	***0.0000***	***0.0000***
QaGA209																		
233	*0.0682*	*0.0510*	*0.0517*	*0.0648*	*0.1000*	*0.0424*	*0.2586*	*0.0395*	*0.0490*	*0.0259*	*0.1071*	*0.1951*	*0.1383*	*0.1071*	*0.0741*	*0.2411*	*0.0119*	*0.1098*
235	***0.0152***	***0.1633***	***0.1897***	***0.2870***	***0.2000***	***0.1271***	***0.2193***	***0.2171***	***0.1961***	***0.1293***	***0.0446***	***0.0976***	***0.0745***	***0.1071***	***0.0556***	***0.0625***	***0.1667***	***0.0488***
241	0.4545	0.3161	0.3276	0.4722	0.4800	0.2288	0.3421	0.2961	0.5784	0.5517	0.6786	0.3293	0.4362	0.3482	0.3889	0.2411	0.6190	0.4390
243	*0.1439*	*0.0204*	*0.0690*	*0.0463*	*0.0400*	*0.1949*	*0.0439*	*0.1447*	*0.0490*	*0.0086*	*0.0625*	*0.0122*	*0.0000*	*0.0000*	*0.0000*	*0.0357*	*0.0000*	*0.0244*
249	***0.0152***	***0.1429***	***0.0345***	***0.0000***	***0.0000***	***0.1441***	***0.0702***	***0.0855***	***0.0539***	***0.1293***	***0.0179***	***0.2683***	***0.1064***	***0.0804***	***0.1111***	***0.2589***	***0.1667***	***0.1098***
251	***0.0303***	***0.0000***	***0.0000***	***0.0000***	***0.0200***	***0.0085***	***0.0000***	***0.0132***	***0.0392***	***0.0086***	***0.0000***	***0.0000***	***0.0127***	***0.1607***	***0.1296***	***0.0357***	***0.0000***	***0.0244***
255	*0.0303*	*0.0204*	*0.0862*	*0.0000*	*0.0400*	*0.0339*	*0.0000*	*0.0329*	*0.0147*	*0.0000*	*0.0357*	*0.0244*	*0.0000*	*0.0179*	*0.0093*	*0.0000*	*0.0000*	*0.0000*

Differentiation statistics computed over all populations are shown in Table 3. All single-locus estimates of among population differentiation (Φ_{ST}) were found to be significantly different from random expectations. Based on stepwise regression analysis, at least one allele at all six of the microsatellite loci were found to be significantly ($p<0.05$) associated with latitude or longitude (see markers in *italic* and ***bold italic***, respectively in Table 2). A visual inspection of allele frequencies across the sample sites shows a northeast-southwest trend. Allele frequencies tend to be either low in the northeast and high in the southwest, or vice versa. Due to this apparent trend, we again performed regression analysis using the composite dependent variable (CDV) that combined both latitude and longitude. Results of these regression analyses are displayed in Table 4. Frequencies of alleles at all six loci were found to significantly vary with the value for CDV. An example of these changes are illustrated in Figure 2 for two alleles, one with allele frequency increasing with CDV distance and the other with allele frequency decreasing. The number of rare alleles per locus was also found to be significantly associated with the CDV for three of the six microsatellite loci (Table 4). A visual inspection of the number of rare alleles across sample sites again shows a northeast-southwest trend, with higher numbers of rare alleles being harbored in southwestern populations. Several loci were also found to be significantly associated with CDV based on the number of unique alleles, effective number of alleles, and gene diversity (Table 4). As a general trend, there appears to be slightly more alleles and hence more effective numbers of alleles and

slightly higher levels of gene diversity in southwest populations than in those located in the northeast (Figure 2).

Table 3. Summary of genetic diversity descriptive statistics for six microsatellite loci segregating in 18 populations of *Castanea dentata* Borkh. located throughout the species natural range in eastern North America.

Locus	Sample Size	n_a[a]	n_e	h	h_o	Φ_{ST}	Nm
*Cs*CAT01	1974	31	9.222	0.892	0.844	0.097	4.655
*Cs*CAT14	1974	15	3.779	0.735	0.710	0.029	16.741
*Cs*CAT15	1336	15	8.519	0.883	1.000[c]	0.032	15.125
*Qa*CA022	1998	13	4.198	0.762	0.730	0.046	10.370
*Qa*GA068	1982	17	7.144	0.860	0.786	0.030	16.167
*Qa*GA209	1936	15	4.456	0.776	0.705	0.034	14.206
Mean	1870	17.667	6.220	0.818	0.755[d]	0.048[b]	12.877
St. Dev		6.653	2.379	0.068	0.059		

[a]n_a = observed number of alleles, n_e = effective number of alleles, and h = Nei's (1978) gene diversity, h_o = observed heterozygosity, Φ_{ST} = Michalakis and Excoffier's (1996) measure of among population differentiation, and Nm = number of migrants exchanged between populations per generation
[b]Mean Φ_{ST} was estimated by summing variance components across loci
[c]observed heterozygosity for this locus was equal to one as the second allele for all trees amplifying only one apparent microsatellite allele was scored as unknown or missing data
[d]Mean and St. Dev. do not include h_o for locus *Cs*CAT15

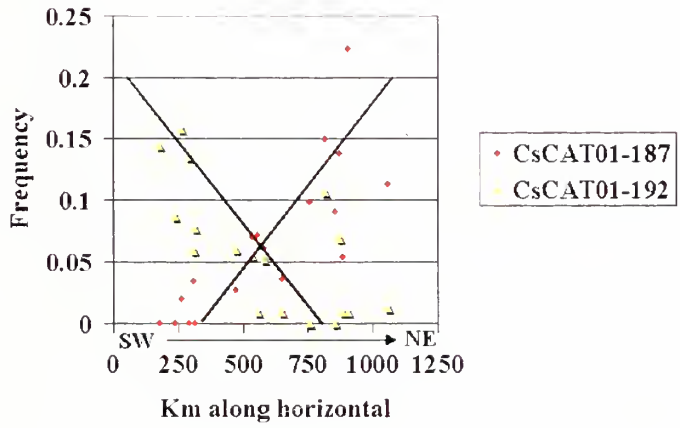

Figure 2. Plot of allele frequency by composite dependent variable (CDV) expressed in units of kilometers along horizontal.

Table 4. Summary of regression analyses for significant associations (Pr>F<0.05) between allele frequency, number of rare alleles, observed number of alleles, effective number of alleles, and gene diversity and a composite dependent variable (CDV) expressed in units of kilometers.

Frequency

Locus	Allele (bp)	Regression equation	R^2	Pr>F
CsCAT01	186	Y=0.07264-0.00005024*CDV	0.391	0.0055
CsCAT01	187	Y=-0.03971+0.00011178*CDV	0.659	<0.0001
CsCAT01	191	Y=-0.01470+0.00006155*CDV	0.397	0.0051
CsCAT01	192	Y=0.13460-0.00008108*CDV	0.490	0.0012
CsCAT01	200	Y=0.08059-0.00004559*CDV	0.330	0.0127
CsCAT14	145	Y=0.00572-0.00000441*CDV	0.304	0.0177
CsCAT15	117	Y=0.10464-0.00006849*CDV	0.335	0.0118
CsCAT15	127	Y=0.05820-0.00003688*CDV	0.337	0.0115
CsCAT15	137	Y=-0.01573+0.00017672*CDV	0.559	0.0004
CsCAT15	139	Y=0.08369-0.00000003*CDV^2	0.248	0.0353
*Qa*CA022	172	Y=0.19220+0.00000007*CDV^2	0.371	0.0073
*Qa*CA022	174	Y=-0.06282-0.00009197*CDV+0.00000003*CDV^2	0.744	<0.0001
*Qa*GA068	164	Y=0.14814-0.00025546*CDV+0.00000011*CDV^2	0.523	0.0039
*Qa*GA068	180	Y=0.04318-0.00002958*CDV	0.309	0.0166
*Qa*GA209	235	Y=0.18213-0.00000005*CDV^2	0.267	0.0282
*Qa*GA209	243	Y=0.12080-0.00007540*CDV	0.352	0.0095
*Qa*GA209	249	Y=-0.01543+0.00008946*CDV	0.257	0.0318
*Qa*GA209	251	Y=0.00268+0.00000003*CDV^2	0.231	0.0436
*Qa*GA209	255	Y=0.04579-0.00002821*CDV	0.317	0.0150
106	525	Y=0.77195-0.00011743*CDV	0.426	0.0045
225	800	Y=-0.07714-0.00033161*CDV	0.494	0.0017
237	1000	Y=0.85531-0.00008368*CDV	0.251	0.0405
237	1250	Y=0.40626-0.00086307*CDV+0.00000037*CDV^2	0.624	0.0011

Number of Rare Alleles[a]

Locus	Regression equation	R^2	Pr>F
CsCAT01	Y=14.18493-0.00490*CDV	0.289	0.0213
CsCAT15	Y=5.13625-0.00331*CDV	0.515	0.0008
*Qa*GA068	Y=8.91562-0.01293*CDV+0.00000513*CDV^2	0.615	0.0008
All loci	Y=58.40985-0.07272*CDV+0.00003079*CDV^2	0.593	0.0012

Observed Number of Alleles[b]

Locus	Regression equation	R^2	Pr>F
CsCAT01	Y=0.65276-0.00079907*CDV+0.00000036*CDV^2	0.548	0.0026
CsCAT15	Y=0.44260-0.00050452*CDV+0.00000022*CDV^2	0.512	0.0046
*Qa*CA068	Y=0.47837-0.00060844*CDV+0.00000027*CDV^2	0.517	0.0043

Effective Number of Alleles

Locus	Regression equation	R^2	Pr>F
CsCAT15	Y=8.79012-0.00212*CDV	0.525	0.0007

Gene Diversity

Locus	Regression equation	R^2	Pr>F
CsCAT15	Y=0.87519-0.00000003*CDV^2	0.463	0.0019

[a]number of rare alleles = number of rare alleles in population/number of individuals in population
[b]observed number of alleles = number of observed alleles in population/number of individuals in population

Estimates of genetic distance (D) between pairwise comparisons of populations based on all six loci varied from a low of 0.062 to a high of 0.372, averaging 0.206. Similarly computed pairwise identity estimates ranged from 0.689 to 0.940, yielding a mean of 0.814. Pairwise estimates of genetic distance were significantly (p=0.0011) associated with the geographic distance between paired populations. However, only a small proportion of the variation found among populations was explained by this dependent variable (R^2=0.069). Estimates of genetic differentiation (Φ_{ST}) between pairwise comparisons of populations varied from a low of -0.003, to a high of 0.156, and averaged 0.048 across loci. These estimates were not significantly associated with geographic distance between the paired populations. Thus, populations in close geographic proximity tend to have slightly higher genetic identities than those

more geographically distant. Single-locus, as well as multi-locus, UPGMA based on genetic distance and PCA based on allele frequencies computed over all sample sites did not reveal patterns of differentiation consistent with regional structure. Geographically proximate sample sites did not group together, and group membership varied from locus to locus.

RAPD-based genetic differentiation

Data describing the RAPD loci used in our analyses are presented in Table 1. In all populations studied, genotypic frequencies observed for microsatellite loci did not significantly deviate from Hardy-Weinberg expectations. Assuming then that the RAPD loci we investigated also have genotypes distributed in Hardy-Weinberg proportions, we can estimate their allele frequencies from observed frequencies for the homozygous null genotypes. Allele frequencies estimated using this approach are displayed by population in Table 5. Sixteen of 19 single-locus contingency χ^2 and G^2 tests for heterogeneity of allele frequencies across populations were found to be significant ($p<0.05$).

Table 5. Band-present RAPD allele frequencies for 19 loci assayed from samples collected in 17 populations of *Castanea dentata* Borkh. located throughout the species natural range in eastern North America. Alleles significantly associated with latitude or longitude are identified in italic and ***bold italic***, respectively.

Locus	CCNC	BCNC	GCSC	PCKY	RCKY	SGCVA	ONTCA	PCWV	GCMD	WCPA	YCPA	MPCPA	UCNY	RCNY	HCMA	MCCT	ME
106_{0500}	0.1308	0.1722	0.0211	0.0658	0.0426	0.1056	0.1762	0.2929	0.1728	0.0839	0.0887	0.0780	0.2421	0.1220	0.1982	0.0364	0.0917
106_{0525}	***0.3084***	***0.1611***	***0.1340***	***0.2138***	***0.1584***	***0.1244***	***0.1136***	***0.1921***	***0.0547***	***0.1036***	***0.0887***	***0.0780***	***0.0324***	***0.1835***	***0.0000***	***0.0000***	***0.1056***
106_{0650}	0.7446	0.6400	0.8000	0.8652	0.5918	1.0000	0.8093	0.8830	0.6181	0.6220	0.8159	0.8419	0.7083	0.7980	0.8110	0.7327	0.7261
106_{0700}	0.0000	0.0474	0.1835	0.0187	0.0646	0.0513	0.0090	0.0138	0.0000	0.0009	0.0087	0.0382	0.0435	0.0000	0.0272	0.0364	0.0126
106_{0800}	0.7051	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.8821	1.0000	0.6727	0.8143	0.8419	1.0000	0.7474	1.0000	1.0000	1.0000
184_{0450}	*0.0221*	*0.0973*	*0.0000*	*0.0090*	*0.0000*	*0.0433*	*0.0000*	*0.0344*	*0.0000*	*0.0000*	*0.0000*	*0.0000*	*0.0000*	*0.0000*	*0.0087*	*0.0000*	*0.0126*
184_{1150}	0.0435	0.0973	0.1340	0.0646	0.1056	0.0980	0.0770	0.0859	0.0786	0.0299	0.0691	0.0000	0.0000	0.2175	0.0087	0.0000	0.0126
184_{1800}	0.1495	0.3061	0.1340	0.1443	0.1835	0.3622	0.2230	0.2289	0.3064	0.1034	0.2042	0.2929	0.3386	0.0632	0.1982	0.3453	0.3481
213_{0900}	***0.2421***	***0.1368***	***0.2929***	***0.1798***	***0.0911***	***0.2254***	***0.1972***	***0.1835***	***0.1815***	***0.1679***	***0.0728***	***0.0968***	***0.2421***	***0.3353***	***0.6526***	***1.0000***	***0.2745***
213_{1060}	0.3477	0.4059	0.4084	0.4606	0.4599	0.2362	0.2112	0.3280	0.3660	0.2279	0.2867	0.5412	0.4947	0.2462	0.2689	1.0000	0.3206
225_{0800}	***0.7446***	***1.0000***	***1.0000***	***1.0000***	***1.0000***	***1.0000***	***1.0000***	***0.8821***	***0.7621***	***0.6331***	***0.4702***	***0.5918***	***0.6703***	***0.4161***	***0.5044***	***0.8333***	***0.6508***
225_{1450}	0.0022	0.0000	0.0000	0.0000	0.0000	0.0000	1.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0897	0.0000
237_{0825}	0.3386	0.2672	0.2546	0.4117	0.2517	0.2362	0.1310	0.1780	0.2327	0.2279	0.1943	0.4689	0.1340	0.0426	0.3841	0.1590	0.2510
237_{1000}	***0.0871***	***0.0093***	***0.1416***	***0.1611***	***0.2789***	***0.0084***	***0.0417***	***0.0921***	***0.0483***	***0.1129***	***0.0177***	***0.0129***	***0.0871***	***0.0105***	***0.0262***	***0.0000***	***0.0247***
237_{1250}	*0.3084*	*0.3914*	*0.3511*	*0.2302*	*0.2517*	*0.1633*	*0.0632*	*0.3175*	*0.1035*	*0.1914*	*0.0823*	*0.0801*	*0.0211*	*0.1248*	*0.1906*	*0.0382*	*0.2510*
423_{0600}	0.1403	0.1835	0.0426	0.1158	0.0835	0.1633	0.1313	0.2536	0.2341	0.2352	0.2277	0.0267	0.1882	0.3773	0.0903	0.2632	0.1377
423_{0875}	0.0109	0.0392	0.0675	0.0180	0.0408	0.0426	0.0190	0.0598	0.0652	0.0801	0.0267	0.0823	0.0114	0.0742	0.0267	0.0000	0.0392
500_{0775}	0.0000	0.0000	0.0000	0.0000	0.0000	0.0084	0.0000	0.0066	0.0246	0.0000	0.0000	0.0000	0.0572	0.0000	0.0457	0.0000	0.0123
514_{0575}	0.1972	0.3412	0.3406	0.2799	0.1282	0.1734	0.2459	0.2494	0.3614	0.3140	0.2277	0.1029	0.0585	0.5337	0.6026	0.2735	0.3753

Differentiation statistics computed over all populations are presented in Table 6. Estimates of among population differentiation (G_{ST}) were found to be significantly greater than zero at 14 of the 19 loci. Based on our stepwise regression analysis, allele frequencies at six of the 19 RAPD loci were significantly ($p<0.05$) associated with latitude or longitude (markers in *italic* and ***bold-italic***, respectively in Table 5). As was observed for the microsatellite loci, a visual inspection of allele frequencies across the sample sites showed a northeast-southwest trend. Again, we performed regression analysis using the CDV. Four loci were found to be significantly associated with the CDV (Table 4). At all four loci, band-present allele frequencies were higher in southwest populations than in those from the northeast.

Estimates of genetic distance (D) between pairwise combinations of populations computed across loci varied from a low of 0.003, to a high of 0.144, with a mean value of 0.037. Similarly computed pairwise identity estimates ranged from 0.866 to 0.997, with a mean of 0.964. Unlike the microsatellite data,

pairwise estimates of genetic distance were not significantly (p=0.0571) associated with the geographic distance between paired populations and neither were pairwise estimates of genetic differentiation. As was the case for the microsatellite loci, single-locus or multi-locus UPGMA computed from RAPD genetic distances, or PCA based on RAPD allele frequencies, did not reveal differentiation patterns suggestive of regional structure.

Table 6. Summary of genetic diversity descriptive statistics for 19 RAPD loci assayed from samples collected in 17 populations of *Castanea dentata* Borkh. located throughout the species natural range in eastern North America.

Locus	Sample Size	n_e	h	G_{ST}	Nm
106_{0500}	845	1.321	0.243	0.049	9.802
106_{0525}	845	1.273	0.214	0.056	8.443
106_{0650}	849	1.552	0.356	0.062	7.628
106_{0700}	844	1.051	0.049	0.056	8.471
106_{0800}	843	1.178	0.151	0.184	2.222
184_{0450}	883	1.030	0.029	0.046	10.364
184_{1150}	881	1.142	0.124	0.050	9.413
184_{1800}	878	1.567	0.362	0.047	10.080
213_{0900}	794	1.535	0.348	0.067	6.914
213_{1000}	801	1.818	0.450	-0.006	2000.0
225_{0800}	808	1.598	0.374	-0.008	2000.0
225_{1450}	810	1.010	0.010	-0.336	2000.0
237_{0825}	871	1.578	0.366	0.060	7.863
237_{1000}	873	1.126	0.112	0.081	5.709
237_{1250}	869	1.416	0.294	0.081	5.642
423_{0600}	861	1.426	0.299	0.053	8.889
423_{0875}	858	1.090	0.082	0.016	30.536
500_{0775}	870	1.021	0.021	0.031	15.406
514_{0575}	858	1.700	0.414	0.093	4.899
Mean	850	1.339	0.226	0.036	9.517[b]
St Dev		0.258	0.148		

[a] n_e = effective number of alleles, and h = Nei's (1978) gene diversity, G_{ST} = Nei's (1987) measure of among population differentiation, and Nm = number of migrants exchanged between populations per generation
[b] Mean excludes estimates for loci 213_{1000}, 225_{0800}, and 225_{145}

DISCUSSION

One of our main concerns in this investigation was inclusion of trees that are not pure American chestnut. Inappropriate trees include interspecific hybrids or pure species other than American chestnut, especially the native congener species chinkapin (*Castanea pumila*). Inclusion of such contaminants could have inflated our estimates of genetic diversity, especially in populations containing the non-American chestnut samples, as well as clouded true patterns of genetic variability. Chloroplast DNA sequence variations have been widely used to investigate interspecific relationships among plant species (Palmer et al. 1988, Clegg et al. 1991) because they evolve slowly. We identified a chloroplast-specific marker (primers a and b; Taberlet et al. 1991) that quickly differentiates American chestnut chloroplast DNA from all other *Castanea* species, including the native *C. pumila*. Unfortunately, maternal inheritance of chloroplasts precludes our ability to distinguish interspecific hybrids of maternal American chestnut origin. As a result, our sample set might still contain some interspecific hybrids, however, the number should be small as most collections were made in either State Forests or National Forests where non-native *Castanea* species do not extensively occur.

Our results demonstrate that high levels of microsatellite and RAPD variability exist in American chestnut, and that most of this variation occurs within local populations (95.2% and 96.4%, respectively). These results are comparable to observations made in other long-lived, outcrossing, woody plant species (Hamrick and Godt 1990; Hamrick et al. 1992), where as a rule, greater than 90% of the variation occurs within populations. Our results are also consistent with previous observations of allozyme variability in *C. sativa* and American chestnut, where 90% of the diversity was reported to exist within populations (Pigliucci et al. 1990; Huang et al. 1998). Whereas only scant evidence for a cline in allele frequency variation (alleles at 1 of 14 polymorphic allozyme loci) was previously reported for American chestnut (Huang et al. 1998), our results clearly demonstrate that a cline in allele frequencies and number of rare alleles exists along the Appalachian axis. Clinal variation of allele frequencies along latitudinal and longitudinal gradients has been reported for a number of tree species (Lagercrantz and Ryman 1990; Zanetto and Kremer; Leonardi and Menozzi 1995, Tomaru et al. 1997), including *C. sativa* (Pigliucci et al. 1990; Villani et al. 1991; Villani et al. 1992; Villani et al. 1994). The main proposition set forth to explain this phenomenon is that geographical variation in allele frequencies resulted from post-glacial migration and founding events. Such processes are consistent with the patterns of variability we observed for American chestnut. The highest levels of gene diversity and the greatest numbers of rare alleles are found in the southwestern portion of its range. This suggests that its glacial refugium existed in the southeastern U.S., perhaps extending southward into the Gulf Coastal plain of present day Mississippi and Alabama. As a general finding, American chestnut still exists as a highly variable species, even at the margins of its natural range, with a large proportion of its genetic variability occurring within populations. Furthermore, existence of the clinal pattern of variation implies that extensive gene flow took place among populations before the spread of chestnut blight.

Although most of the genetic variation found in American chestnut occurs within local populations, a statistically significant proportion exists among populations. Magnitudes of the Φ_{ST} and G_{ST} estimates obtained in our investigation are slightly lower than those reported for American chestnut by Huang et al. (1998). In this research we used a chloroplast-specific marker to identify trees that were not pure American chestnut and excluded these individuals. However, Huang et al. (1998) did not take precautionary measures to identify aberrant specimens. Inclusion of such individuals in some samples will tend to inflate levels of among population differentiation. Although our estimates of among population differentiation might be considered low, Φ_{ST} values obtained for all six microsatellite loci and G_{ST} values obtained for 14 of the 19 RAPD loci studied indicate that populations significantly differ in allele frequency. Moreover, population pairwise estimates of genetic distance, based on microsatellite haplotype frequencies, were shown to be significantly associated with the geographic distance between populations. Thus we conclude that geographically proximate populations are slightly more genetically similar than geographically distant populations. These findings lead us to conclude that although long distance gene flow was possible in the past, it was infrequent enough to allow genetic differentiation to take place.

From UPGMA and PCA analyses, it is evident that regional differentiation did not occur in American chestnut. Geographically proximal populations did not group together, and group make-up differed across loci. In contrast, Huang et al. (1998) concluded that a somewhat weak and incomplete pattern of regional differentiation exists, based largely on latitudinal differences. Although the results obtained by UPGMA and PCA of the allozyme data were interpreted as being suggestive of regional structure, hypothetical regional effects were not quantified by means of a hierarchical AMOVA, nor were statistical tests employed to detect differences. Because of our more comprehensive sampling of the natural range (18 populations versus 12), larger sample sizes collected (average 55 trees per population versus 22 trees), and elimination of suspect samples (i.e. trees that did not have the characteristic American chestnut chloroplast haplotype), we believe the results obtained in this investigation represent a more accurate picture of population structure in American chestnut.

Our findings clearly demonstrate that American chestnut still exists as a highly variable species throughout its entire native range. In spite of this high variability, we must point out that the results of this study represent variability existing in the pre-blighted forest, and caution that unless measures are taken to restore American chestnut and enhance opportunities for it to sexually reproduce, this species will likely face serious erosion of its gene pool as root systems fail to produce sprouts and die. Along these lines, results of this study can be used as a baseline in the future for assessing the degree and rapidity of such a decline.

Taking into account the differentiation observed at these loci, no disjunct regional pattern of variation exists. Prior to introduction of the blight, genetic variability in American chestnut followed a pattern consistent with the hypothesis of a single metapopulation where genetic drift played a major evolutionary role. Currently, approximately 95% of the neutral genetic variation of the species can be captured by sampling within any one population. However, the results of this study are based on neutral genetic loci and do not necessarily reflect genetic differentiation for adaptive genes or gene complexes. Therefore, in order to assure that most of the variation produced by these genes is also captured in conservation and breeding endeavors, sampling should focus on collecting a fairly large number of individuals (50 to 100 or more) from each of several geographic areas.

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REGIONAL ADAPTATION IN AMERICAN CHESTNUT

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Abstract: Conservation of forest genetic resources, such as restoration of American chestnut, requires knowledge of genetic variation patterns in adaptive, non-neutral alleles. Almost no such information is available for American chestnut, but there is information available from other forest tree species including species that are sympatric with chestnut. Some of that information is summarized in this paper, and the adaptive significance of several growth and physiological characteristics is discussed. Most tree species exhibit "racial" patterns of genetic variation that parallel geographic gradients in climate. Wild populations that have survived in a locality for many generations have a genetic identity of place that reflects a history of natural selection and adaptation. Judging from genetic variation patterns in sympatric species, American chestnut populations are probably genetically distinct in important and somewhat predictable ways. American chestnut breeding and restoration projects should be guided by this knowledge.

Keywords: genetic / geographic / racial / variation / selection / adaptation / growth rate / cold tolerance / phenology

INTRODUCTION

With new technologies for controlling chestnut blight on the horizon, we are beginning to contemplate the restoration of American chestnut to something like its former importance in our forests. The creation of genetically resistant trees through breeding or genetic engineering is especially promising as a foundation for restoration efforts. However, blight-resistant alleles can be introduced to (or found in) only a tiny fraction of the chestnut trees that still survive, so any restoration program that employs blight-resistant trees will inevitably force the species through a genetic "bottleneck" with the danger that important alleles may inadvertently be lost. Like other plant species, especially those with large natural distributions, American chestnut undoubtedly contains a great deal of genetic variation. This diversity should be protected (Irwin 2003), and indeed it should be exploited if possible in the restoration process itself. But to do so will require an understanding of how genetic variation is structured within the species.

Information about range-wide genetic variation in American chestnut is limited to studies of allozymes and DNA markers of unknown and probably neutral adaptive significance (Huang et al. 1998, Kubisiak and Roberds 2003). These studies revealed that differences among populations account for only 5 to 10 percent of the total genetic variation measured, results that closely resemble the findings of other studies of neutral alleles in species that are similar to American chestnut in mating system, longevity, population size and density, and other characteristics that affect gene flow (Hamrick and Godt 1990). This is useful knowledge – for example, it tells us that gene flow among populations has been relatively strong within this species – but it tells us virtually nothing about the structure of genetic variation in alleles subjected to the pressures of natural selection. Patterns of variation in adaptively neutral genetic markers may bear little relationship to patterns of variation in adaptively relevant alleles, whose variation patterns may also differ from one another according to what kind of characteristics they control (Morgenstern 1996). Genetic variation in "fitness" characteristics (in the terminology of Darwinian theory), especially those responding to regional selection gradients such as climate, is highly relevant to conservation or restoration efforts undertaken at a regional or range-wide scale.

Unfortunately, we know almost nothing about genetic variation in fitness characteristics within American chestnut. There is, however, a fairly substantial body of such information from studies of other tree species, and this knowledge can be used to inform future decisions about American chestnut. This literature comes from replicated, "provenance" tests of progeny from natural populations grown in a common environment. Such experiments, if properly designed, permit the researcher to apply the methods of quantitative genetics to measure and test contributions of genetic variation to phenotypic variation, even without knowing the underlying DNA structure or mode of gene action. It is even possible to partition the relative contributions of among- versus within-population genetic variance just as population geneticists do when working directly with DNA markers. In this paper I provide some examples of such research on eastern forest tree species. Although we cannot know for certain, American chestnut would probably exhibit similar genetic variation over similar environmental gradients if it were studied in the same way.

EXAMPLES OF REGIONAL VARIATION IN ADAPTIVE CHARACTERISTICS

Bud-Burst Timing in Eastern American Species

Deciduous trees can take advantage of abundant moisture and shade-free conditions (in the case of plants growing below the forest canopy) by initiating growth early in the spring, but early growth initiation increases the risk of frost injury to young leaves. Thus, genetic control of bud-burst timing is probably under strong selective pressure for optimality in any given environment. It is typically the case in common garden tests that populations from colder (more northern or higher elevation) environments burst bud earlier in the spring. Bud burst in trees is usually cued by rising temperature, and populations from colder climates are adapted to grow (and begin growing) under cooler temperatures. When the geographic pattern of bud-burst timing is the opposite (*e.g.*, southern populations earlier in common gardens), as has been recorded in a few species, it is likely attributable to a different environmental cue for growth (photoperiod) rather than a fundamental difference in the way the plant has adapted to environmental gradients (Steiner 1979a). Of course, in nature, plants in warmer climates always begin growing before plants in colder climates. But if the onset of spring is heralded by the appearance of leaves on trees, spring would be even more delayed in the north if all populations of a species required equally warm temperatures for growth.

Steiner (1975 and 1979b) described geographic patterns of genetic variation in bud-burst timing in three species that are broadly sympatric with American chestnut: yellow birch, eastern white pine, and Virginia pine. The first two species are sympatric with chestnut throughout most of its Appalachian distribution, but they also occur widely in the Lake States and southeastern Canada. Virginia pine occurs naturally only from central Pennsylvania southward, but the whole of its distribution is very similar to the southern half of American chestnut's. All three species showed the typical north-early / south-late pattern of variation in bud-burst timing in common-garden tests. Also, all three exhibited genetic variation within their area of sympatry with chestnut, with clinal gradients statistically detectable over minimum distances of 100 to 300 km. A partial exception was yellow birch, which showed no clear latitudinal gradient in bud-burst timing in from Pennsylvania southward (roughly the southern half of the American chestnut range). Steiner (1975) also found that population variation in time of flowering (pollen release) generally corresponded with population variation in bud-burst timing. This may not be the case with the late-flowering American chestnut, whose habit of flowering in mid-summer may not be so closely linked in both a physiological and genetic sense with its phenology of vegetative growth.

Broad climatic gradients in genetic variation are almost always somewhat muddled by populations that do not fit the trend, and this was true in Steiner's studies. Elevations of origin differed greatly for the

Appalachian populations of all three species, and it is reasonable to suppose that adaptation to elevation might have explained locally "anomalous" populations. However, there was no detectable relationship between elevation and bud-burst timing, at least after accounting for latitude (the more southern populations tended to occur at higher elevations). A better test of the effect of elevation of origin on genetic variation would be to deliberately sample a number of populations along an elevational transect up and down a single mountain. I am not aware of any such study in the Appalachians, but McGee (1974) studied four populations of northern red oak collected from different elevations "within 100 km of Asheville, North Carolina" and found a possible elevational effect on genetic differentiation in bud-burst timing in that species. From these studies we can predict that American chestnut seed that is moved to environments that are warmer or colder than the native environment will likely be somewhat "off" in the timing of new growth in the spring, growth cessation in late summer, and perhaps flowering.

Genetic Variation in Cold Tolerance in Two Species

The process of acclimation to cold in woody plants begins after the cessation of growth and is triggered by diminishing day length. Acclimation deepens as plants experience increasingly colder temperatures, reaching a maximum when temperatures are coldest, in January or February. This process has a metabolic cost, and plants that develop greater levels of cold tolerance presumably pay a price for that advantage. The benefit to a plant of adequate cold tolerance, and the cost of its absence, is clear and direct. Not surprisingly, geographic patterns of genetic variation in ability to acclimate to low temperature tend to look very much like January low-temperature isotherms on a map.

Williams (1984) described a nicely done study of genetic variation in cold tolerance within green ash. Green ash has a native range that extends far beyond the region in which American chestnut grows, but Williams' study included seven populations of green ash from the area of overlap with our species of interest. These populations differed in the expected fashion: the rapidity of acclimation and depth of mid-winter cold tolerance were greatest in New York and Pennsylvania populations, intermediate in central Virginia populations, and least in eastern Tennessee populations. These represented nearly half of the total range of variation in mid-winter cold tolerance levels for all green ash populations studied (which included Manitoba and South Dakota provenances, but none more southern than Tennessee). Williams also found significant *within*-population genetic variation in cold tolerance – except in populations near the northern limit of the range, where the species is presumably at its limit of adaptation to cold.

The results described above were obtained under controlled, laboratory conditions using twigs taken from trees grown out-of-doors. Williams (1984) also measured actual winter injury over a three-year period in nine replicate plantings of a green ash provenance test in the upper Midwest and Northeast. As one would expect, trees that had originated from progressively warmer climates had progressively more severe winter injury. Put another way, the fraction of trees that escaped winter injury diminished as population origin went from north to south. Interestingly, Williams found that some trees escaped injury when growing in environments colder than where they originated, sometimes even much colder, but moving a population to colder environments was *always* accompanied by an increase in the risk of winter injury as measured by percentage of trees injured.

The mating systems, life history characteristics, and population structure of American chestnut and many other forest tree species tend to promote gene flow, which acts to minimize genetic differentiation between nearby populations (Loveless and Hamrick 1984). There are very few known instances of clearly adaptive genetic differentiation in temperate forest trees occurring over distances of a few kilometers or less. One example is that described by Berrang and Steiner (1986) and Steiner and Berrang (1990) for cold tolerance variation in pitch pine. Pitch pine and American chestnut have almost identical

distributions and often co-occur on the same sites. Near State College, Pennsylvania, pitch pine is common within an area called the "Barrens," which often (and during any month of the year) has substantially lower nighttime temperatures than the surrounding countryside. Pitch pine also grows in areas near the Barrens that have locally normal temperatures. These authors compared the development of cold tolerance in dormant seedlings raised under controlled conditions but originating from Barrens and non-Barrens trees. Compared to the neighboring population on normal sites, Barrens seedlings acclimated more rapidly in the fall, achieved greater levels of cold tolerance in mid-winter, and de-acclimated more slowly in the spring. Evidently, differences in selection pressure over the distances separating these populations (about 8 km) have been strong enough to create genetically distinct populations.

Genetic Variation in Height Growth Rate in Northern Red Oak

Everyone knows that plant growth is greatly influenced by environmental conditions, but growth rate is always under genetic control, as well. Growth rate is fundamentally important to plant fitness, though rapid growth is not always (or even usually) the best strategy for ecological success. As Grime (1979) has pointed out, there is essentially a "zero-sum" relationship between plant investment in growth, reproduction, and defense or toleration of stress – the benefits vary according to circumstances, but the costs are always there, and a plant cannot afford to excel at everything. In range-wide provenance tests of species whose distributions span warm/cold or wet/dry climatic gradients, it is typically the case that populations from warmer or moister environments are genetically capable of faster growth than is found in their poor relations living at the limits of hospitable conditions (Wright 1976). This pattern probably arises because competition is a stronger selective force in the milder climates (favoring rapid growth), and stress is a stronger force in the harsher environments, where investment in cold tolerance or drought tolerance or avoidance (always at some metabolic cost) is more advantageous than producing more foliage.

However, this generalization applies most particularly to species that inhabit a truly wide range of environments. Through the smaller and more homogenous region occupied by American chestnut, patterns of genetic variation in growth rate have typically had a strongly "random" character in provenance tests, usually defying simple geographic description. The occurrence of apparently random variation does not necessarily mean that the controlling alleles are selectively neutral, but it may mean that the forces that favor or disfavor rapid growth are more local than regional in occurrence.

Northern red oak occupies the same region as American chestnut plus a little more, occurring from the Gulf Coastal Plain (but not Florida) to eastern Kansas and southern Ontario. Several genetic tests of this species (summarized and reanalyzed by Steiner 1998) were designed to permit the partitioning of genetic variation into "local" and "regional" components. In tests that included populations no more than a few hundred kilometers apart, virtually all of the genetic variation in growth rate occurred *within* populations (populations differed little or not at all). However, even in a "range-wide" test (with distances between populations of up to 2000 km), within-population genetic variance still accounted for 64 percent of total genetic variance in growth rate. Furthermore, variation *among* northern red oak populations in growth rate, when it occurs, does not show a clear and straightforward geographic pattern (Steiner 1998). Steiner argued that selective forces acting on growth rate in northern red oak may plausibly operate on a very local, "microsite" scale in stands of this species. (Forest soils are typically very heterogeneous *vis-à-vis* growth potential in northern red oak.) If American chestnut has a similar pattern of genetic variation, then a typical population may contain most of the genetic variation that occurs within the species in alleles controlling growth rate. However, the possibility of important, inter-population variation should not be ignored.

CONCLUSIONS

We know almost nothing about genetic variation in characteristics of American chestnut that play a role in adaptation to the environment. However, most tree species exhibit "racial" patterns of genetic variation that parallel geographic gradients in climate. Wild populations that have survived in a locality for many generations have a genetic identity of place that reflects a history of natural selection and adaptation. When environmental differences are large enough, natural selection may favor genetic differentiation even on a rather local scale. Studies of other species have consistently revealed genetic variation – within the species' region of sympatry with American chestnut – in adaptively important characteristics such as growth rate, phenology, and cold tolerance. Disrupting these variation patterns by careless human meddling can result in trees that are unsuited to their environments in subtle but perhaps important ways, particularly considering that trees with normal lifespans must survive many decades of environmental vicissitude. In the absence of evidence to the contrary, we should expect that American chestnut populations also differ genetically from one another in similar ways. This knowledge should guide breeding and restoration projects in American chestnut. Restoration projects should seek to preserve as much natural genetic variation as possible within American chestnut, and blight-resistant trees used to restore wild populations should be derived from locally or regionally native American chestnut trees.

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RATE OF RECOVERY OF THE AMERICAN CHESTNUT PHENOTYPE THROUGH BACKCROSS BREEDING OF HYBRID TREES

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Abstract: We describe a study in which the morphological characteristics of several chestnut populations – American chestnut, Chinese chestnut, first-generation hybrids, and first-, second-, and third-generation backcross hybrids – were quantified and compared. Twenty-four morphological variables known to distinguish American and Chinese chestnuts were used to develop a composite Index of Species Identity. The aggregate morphology of the first hybrid generation was almost exactly intermediate (mean ISI = 0.50) between Chinese (0.11) and American chestnut (0.85). The first backcross generation resembled American chestnut more than expected, but the second and third backcross generations conformed closely to expectations. Although some degree of "Chinese" character could be found within the third backcross generation, 96 percent of the trees in this population fell within the range of ISI values for pure American chestnut and none fell within the range of Chinese chestnut.

Keywords: *Castanea dentata* / backcross breeding / morphology / morphometrics / Index of Species Identity

INTRODUCTION

Nearly a quarter of a century ago, Burnham (1981) proposed the backcross breeding system that is currently the basis for The American Chestnut Foundation's (TACF) chestnut restoration efforts. In a few years, TACF expects to produce a third intercross generation from the third generation of backcrosses to American chestnut (Hebard 2002). This BC_3-F_3 generation is expected to be highly blight resistant yet essentially "American" in all other characteristics, and it is expected that these trees will be the basis for the first serious efforts to restore American chestnut to its former habitats in the Appalachian region. Although experience with breeding other plants suggests that the third backcross is sufficient to recover the characteristics of the recurrent parent (American chestnut in this case), no one has yet quantified how well this will work in chestnut breeding. This is an important question for those whose interest is ecological restoration of *American* chestnut. How truly "American" will these trees be?

In this paper we summarize a study that was designed to answer this question by comparing the morphological characteristics of American chestnut, Chinese chestnut, their first-generation hybrid (F_1), and three successive backcross generations to American chestnut (BC_1, BC_2, and BC_3) (Diskin 2003). The morphology of the third backcross generation will be discussed in particular detail because this generation has the same relative proportion of the American chestnut genome as those trees that are currently proposed for use in restoration trials.

OVERVIEW OF THE METHODOLOGY

Twenty-four morphometric variables based on leaf, twig, bud, and stipule characteristics that distinguish American chestnut from Chinese chestnut were measured on trees sampled from TACF's Glenn C. Price Research Farm in Meadowview, Virginia. Approximately 50 trees, ranging in age from two to six years

old, were sampled from each of the following generations: American chestnut, Chinese chestnut, and F_1, BC_1, BC_2, and BC_3 hybrid generations. All 24 variables were measured on each tree, and the results of the individual measurements were analyzed using standard statistical methods.

The overall morphology of each tree was summarized in an "Index of Species Identity" (ISI). The ISI is the score of the first principal component, transformed to a scale from 0 to 1.0, from a principal components analysis of the 24 original variables. Essentially, ISI is a composite index of the best of the variables typically used by taxonomists to distinguish *Castanea dentata* (Marsh.) Borkh. from *C. mollissima* Blume. ISI score frequencies were plotted for each population, and the degree of overlap or separation in frequency distributions was used to compare the aggregate morphologies of the hybrid generations and their parental species. Mean ISI scores were also calculated for each generation and analyzed using standard statistical methods.

MORPHOLOGY OF THE HYBRID GENERATIONS COMPARED TO THEIR PARENTAL SPECIES

Because we measured only variables with proven utility in distinguishing Chinese and American chestnut specimens, the two species occupied the extremes of morphologies observed in the study. Chinese and American chestnuts scored at opposite ends of the scale for each individual variable as well as the composite variable, ISI (mean scores of 0.11 and 0.85 for Chinese and American chestnut, respectively).

Comparative morphologies of the four hybrid generations and their parental species are most easily summarized by ISI scores. The morphology of the F_1 generation was almost exactly intermediate between American and Chinese chestnut, with a mean ISI of 0.50. The first-, second-, and third-generation backcross hybrids were different from the F_1 hybrid but, surprisingly, similar to one another, with ISI means of 0.78, 0.77, and 0.79, respectively.

Expected ISI scores can be calculated for each backcross generation assuming that observed ISI scores for the two species are accurate and assuming a straightforward 50 percent dilution of Chinese alleles in each backcross generation and quantitative, additive inheritance of ISI values. Under these assumptions, the F_1 should have an ISI that is exactly intermediate (0.48) between the parental species, and the observed value of 0.50 is not significantly different from expectation. The BC_1, BC_2, and BC_3 backcross hybrids should have ISI means of 0.67, 0.76, and 0.81, respectively, or halfway toward the American species value of 0.85 in each successive generation.

Although ISI values for the BC_2 and BC_3 populations were similar to one another, they did not differ significantly from the values expected under the above assumptions (0.77 vs. 0.76 and 0.79 vs. 0.81 for BC_2 and BC_3 populations, respectively). The small difference between these two generations simply reflects the fact that backcrossing yields diminishing returns with each generation. However, the BC_1 population was anomalously more similar to pure American chestnut than expected (0.78 vs. 0.67). Among other things, the anomaly may be attributable to the fact that the 48 trees representing this generation were derived from crosses between only one Chinese and two American chestnut parents. Just as one or two individuals may not be representative of an entire species, their progeny may not be representative of a typical hybrid population. (It should be noted that the hybrid populations used in this study are not the same as those used by TACF to produce its BC_3F_2 hybrids, nor are they directly related to one another in the sense, for example, that the particular BC_1 population in this study was used to produce the BC_2 population that we measured.)

Based on ISI values, 90 percent of the BC_2 trees and 96 percent of the BC_3 trees had aggregate morphologies within the range of American chestnut values. None of the trees in these two populations

had the highest ISI values found in a very few American chestnut trees, and a small percentage had values lower than observed in any American chestnut trees. However, no backcross hybrid trees had values even close to the highest ISI values recorded for Chinese chestnut.

American chestnut morphology was fully recovered in the BC_3 generation for 15 of the 24 individual morphological characteristics that were measured. In each of these variables, there was no statistically significant difference between American chestnut and BC_3 trees: leaf relative length, tooth length, tooth depth, leaf length to tooth length ratio, leaf width to tooth depth ratio, lenticel width, bud length, bud yaw angle, tooth hooking, leaf apex shape, interveinal leaf hairs, stipule size, twig color, twig hair density, and bud color. The BC_3 generation did not fully resemble American chestnut in distance from base to maximum leaf width, twig diameter, bud width, bud relative length, bud appression, bud pitch angle, leaf base shape, leaf veinal hair density, and bud tip shape. However, for all but one of these variables (bud relative length), the third backcross generation more closely resembled American chestnut than Chinese chestnut.

CONCLUSIONS

Progress towards American chestnut morphology generally conformed to expectations based upon the proportion of American chestnut genome in the various hybrid generations: the F_1 was almost exactly intermediate between the parental species and the BC_3 was very close to 15/16ths "American" on the composite index scale. Thus, backcross breeding appears to substantially recover American chestnut morphology in the backcross generations. Each of the three backcross generations was distinct from Chinese chestnut in that no individuals fell within the range of Chinese chestnut morphology, but each generation overlapped in morphology with American chestnut. Although the morphology of the third-generation backcross hybrids was largely similar to American chestnut, some Chinese-like characteristics remained. These could probably be further removed through selection for particularly "American" individuals in TACF's BC_3-F_2 generation before the production of BC_3-F_3 seed.

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SELECTION FOR CHINESE VS. AMERICAN GENETIC MATERIAL IN BLIGHT RESISTANT BACKCROSS PROGENY USING GENOMIC DNA

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Abstract: The American chestnut (*Castanea dentata* [Marshall] Borkhausen) was historically one of the most important hardwoods in North America due to its abundance and the multiple functions it served for both ecosystems and humans. The exotic chestnut blight fungus has eliminated the American chestnut as an overstory tree in eastern forest ecosystems, however. Backcross breeding shows promise to produce chestnuts that combine the blight-resistance that evolved in Chinese chestnut (*C. mollissima* Blume) with the desirable characteristics of American chestnut as a forest tree. In the backcross program, blight-resistance is introduced by an interspecific cross of American chestnut with resistant Chinese chestnut trees. American chestnut characteristics are then regained by a series of backcrosses to American chestnut parents. To accelerate and improve this selection process, we developed a molecular protocol to determine the amounts of American vs Chinese chestnut genome among progeny selected for blight resistance. The dot blot technique involves the hybridization of labeled Chinese chestnut genomic DNA to DNA from individual backcross progeny trees, which reveals the amount of Chinese chestnut DNA that remains in them. On average, progeny in the third backcross (BC3) generation should show lesser amounts of hybridization to Chinese chestnut genomic DNA probe than F2, BC1 and BC2 progeny. Because there will be variation among individuals in each backcross generation for the amount of Chinese chestnut genome that they contain, those blight resistant progeny with greater amounts of Chinese chestnut content can be identified by this approach and eliminated from the crossing program. The effectiveness and reliability of this approach are demonstrated using samples from the parents and progeny in three backcross generations.

Keywords: Backcross generations, dot-blot hybridization , genomic DNA, selection

INTRODUCTION

American Chestnut and the Chestnut Blight

Before the introduction of the chestnut blight disease, the American chestnut (*Castanea dentata* [Marshall] Borkhausen) was one of the most important trees in hardwood forests of the eastern United States. With a range centered on the Appalachian Mountains and extending from Maine west to Michigan and south to Alabama and Mississippi (Little 1976), the American chestnut grew in mixtures with many other species, and often comprised 25 percent or more of the hardwood tree population within any given forest stand (Braun 1950).

The American chestnut may have been the most important hardwood in eastern North America due to its abundance and the multiple functions it served for both ecosystems and humans (Hardin *et al.* 2001). It was a dominant component of much of the eastern hardwood forest, and it produced a regular and bountiful nut crop that was an important part of the diet of many animals (Rice *et al.* 1980). Historically, the American chestnut was an important tree because of the assortment of services and commodities it provided to people as well. It was an extraordinary tree for wood fiber production due to its large size, fast growth, and ability to sprout from stumps (Detwiler 1915). American chestnut wood fulfilled a multitude of needs ranging from construction and furniture lumber, firewood, fence construction, railroad

ties, telephone and telegraph poles, pulpwood, and tannins. The chestnuts were also important as a food source for rural residents, and the tree was widely planted to provide shade (Buttrick 1915).

Chestnut blight was first introduced to North America in 1904. The chestnut blight disease is caused by *Cryphonectria parasitica* (Murrill) Barr (=*Endothia parasitica* [Murrill] P.J. and H.W. Anderson), an exotic fungus from Asia that enters through wounds in the bark and eventually girdles the tree, killing susceptible individuals (Roane *et al.* 1986). Because American chestnut trees evolved in the absence of the fungus, they lacked entirely any genetic protection from the fungus (Stiles and Hebard 1996). By 1950 the disease had spread across the entire native range of the American chestnut, eliminating it as an overstory tree in eastern ecosystems (Newhouse 1990). The American chestnut continues to survive as a shrub, however, sprouting from the root collars of stumps in the forest (Hardin *et al.* 2001).

Backcross Breeding Program

The American Chestnut Foundation's (TACF) approach to developing the most resistant trees with the best American characteristics – "*the path of most resistance*" – is shown in Figure 1. After the chestnut blight fungus was introduced to the United States, plant explorer Frank Meyer discovered the fungus in Asia, along with Chinese chestnuts (*C. mollissima* Blume) that had evolved resistance to the disease (Fairchild 1913). Because of the blight resistance of Chinese chestnut, and cold hardiness, this species was selected for developing blight-resistant hybrids with American chestnut that could replace the disappearing (Burnham 1987) in American forests.

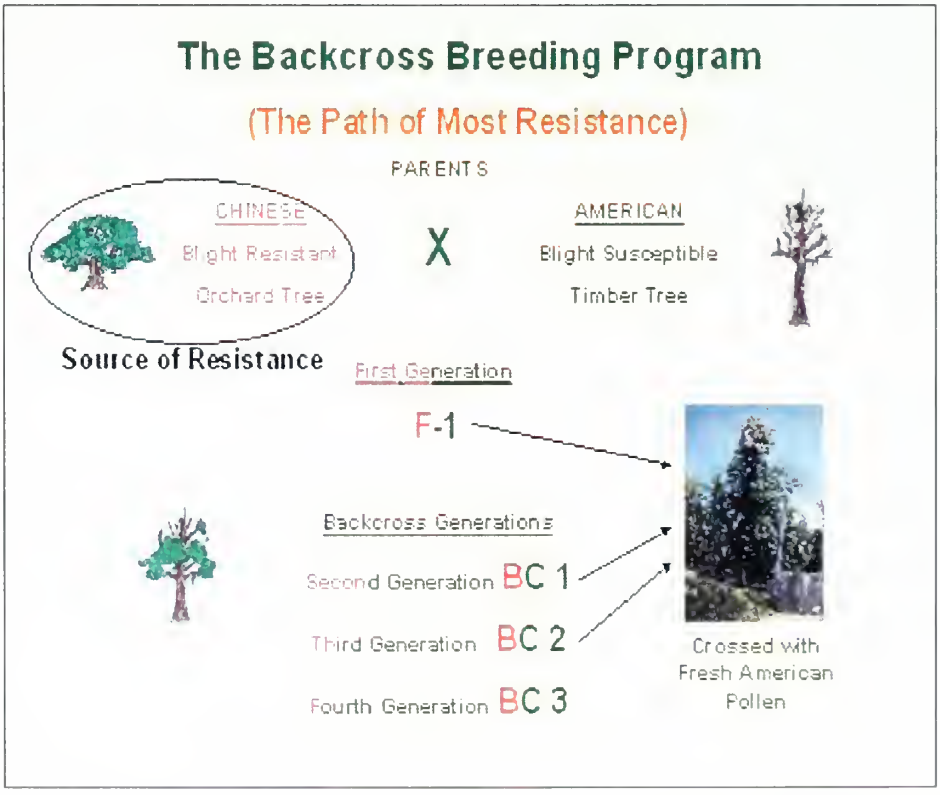

Figure 1. The scheme for the backcross breeding program being used by the American Chestnut Foundation (from Hebard, Http:// chestnut.acf.org).

However, while Chinese chestnut is highly resistant to the chestnut blight, it has other characteristics that make it undesirable as a replacement for the American chestnut. Whereas the American chestnut grows

straight and tall and was formerly a canopy tree species, the Chinese chestnut has a low-growing, sprawling form similar to that of an apple tree. Additionally, American chestnut trees have higher quality timber, sweeter nuts, and a faster growth rate (Hebard 1994a; Stiles and Hebard 1996). The genetic material of the American chestnut also reflects thousands of years of co-evolution with eastern hardwood forest ecosystems. During this time, complex relationships presumably evolved between the American chestnut and other components of the forest, a history that is borne in the genome of the American chestnut (Stiles and Hebard 1996). Thus a program based on back-cross breeding (Figure 1) was developed to recover the American characteristics while retaining the Chinese blight resistant genes.

RATIONALE AND APPROACH

The process of recovering the American characteristics by diluting out all of the Chinese donor parent characteristics, except for blight resistance, usually entails several generations of backcross breeding to recurrent parent trees (AC). The first hybrid generation (F_1) produced by crossing American chestnut with Chinese chestnut inherits one half of its genes from the American chestnut parent and one half from the Chinese parent. These first-generation hybrids are then backcrossed to an American chestnut parent, producing a first backcross generation (BC_1) that has a genome that is on average three-quarters American chestnut and one-quarter Chinese chestnut. Each successive backcross reduces the Chinese fraction of the genome by one-half: the second backcross generation (BC_2) is on average one-eighth Chinese chestnut, and the third and final (in the plan outlined by Burnham) backcross generation (BC_3) is on average fifteen-sixteenths American chestnut and one-sixteenth Chinese chestnut (Rutter and Burnham 1982). However variation occurs among individuals in each backcross generation for the amount of Chinese chestnut genome that they contain due to chromosomal recombinations that naturally occur at gamete formation. In addition, TACF produces intercross (F2) generations (Figure 2) that increase the number of progeny at each generation, and provide greater genetic variation and greater opportunity for blight resistance to be separated from other tree characteristics. Selection for blight resistance and

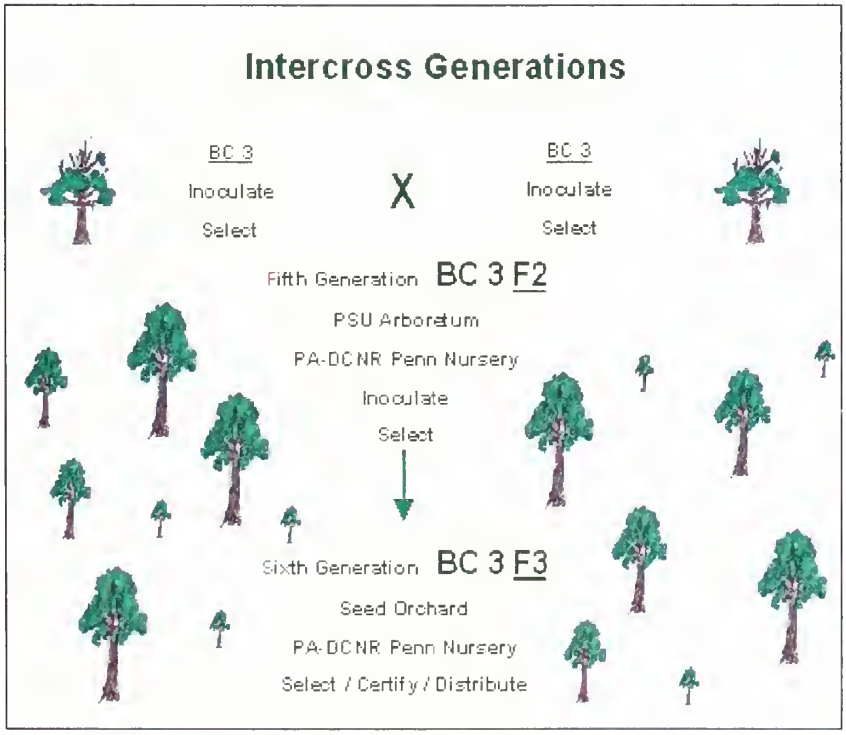

Figure 2. Advanced generation intercross scheme of the American Chestnut Foundationn for seed orchard development and production (http:// chestnut.acf.org)

tree characters is made at each breeding step, which requires intervals of several years. The breeding program could be accelerated through the use of genomics tools for the identification of trees carrying larger portions of American genome at each step, thus also improving the results of each stage of selection.

Many decades of breeding research by the U.S. Department of Agriculture, the Connecticut Agricultural Experiment Station (CAES), and the American Chestnut Foundation indicate that resistance in the Chinese species is carried on two or three genes, which are only incompletely dominant. To achieve full resistance, all the genes from American chestnut that control response to the blight must be replaced by the Chinese alleles. The ACF breeding program has already reached the third backcross generation which is being evaluated in extensive field tests in several states for durability of resistance and for the American tall-timbered growth habit and regional adaptability. Overall. TACF has more than 11,000 trees at various stages of the blight resistance breeding process at its farms in Virginia.

Random amplified polymorphic DNA (RAPD) and restriction fragment length polymorphism (RFLP) markers have been used to construct genetic linkage maps and identify genomic regions (QTLs) conditioning resistance in an F2 population derived from the 'Mahogany' resistance source (Kubisiak et al. 1997). Two of theses regions have since been confirmed in a BC1 population derived from 'Nanking' and PI 34517 suggesting that some of the genomic regions conditioning resistance are syntenic across the different sources (Kubisiak, unpublished). AFLP markers were subsequently been found that flank the blight resistance QTLs and that could thus also be used to select for those loci in progeny (Sisco. unpublished).

However, while individual RAPD, RFLP, and AFLP markers will be good for early selection for the major resistance loci, such linked markers and associated maps with DNA markers will be difficult to use efficiently to select against the chromosomal material from Chinese chestnut that is not associated with resistance. To select against the Chinese genetic background, it will be necessary to use many markers covering all of the linkage maps simultaneously. When many markers are being used in concert, the inherent problems with reproducibility of RAPD markers, with dominance of the AFLP and RAPD markers, and with length of time and inconvenience needed to use RFLP markers would make selection against Chinese genetic background by the DNA marker approach very complicated. and quite expensive.

We have developed a simple dot blot protocol to rapidly screen individual trees in the breeding program for their content of American versus Chinese chestnut genome. The technique involves the hybridization of labeled Chinese chestnut DNA to the DNA of individual trees, using American chestnut DNA to block the detection of sequences shared by American and Chinese chestnut.

In the present study we tested the effectiveness and reliability of the dot blot technique to directly select against the Chinese genome in progeny of the BC3 generation. On average, progeny in the BC3 generation should show lesser amounts of hybridization to the Chinese chestnut genomic DNA probe than F2, BC1 and BC2 progeny. It should also be possible to identify those blight resistant progeny within each backcross generation with greater amounts of Chinese chestnut content by this approach, so that they can be eliminated from the crossing program.

The second objective of this study was to evaluate how closely the data from the dot blot protocol correlated with visual evaluation of known morphological characteristics. We assume that the variation of hybridization of American genomic DNA among individuals within each BC generation is coincident with the variation of the morphological characteristics that taxonomically distinguish American chestnut and Chinese chestnut. In the study of variation of the morphological characteristics among individuals within generations conducted by Matt Diskin (2003, and previous chapter in this proceedings), twenty-four morphometric characteristics known to discriminate between American and Chinese chestnut were

measured on each of approximately 50 individuals in the parental species, the first-generation hybrids, and in each of the three backcross generations. Principal components analysis was used to develop an Index of Species Identity (ISI) that described the aggregate morphology of the different populations. As expected, the morphologies of American and Chinese chestnut were the extremes measured in this study. The first-generation hybrids were intermediate between the two parental species, and the three backcross generations had similar morphologies, distinct from Chinese chestnut and largely similar to American chestnut. American chestnut morphology was essentially recovered in the third backcross generation, for the 24 characters studied. To find the relationship between variation in hybridization data and morphology, DNA was obtained from the sample individuals used in the morphology study.

MATERIALS AND METHODS

Chestnut Materials and Sample Selection

Tissue samples were collected by Matthew Diskin (undergraduate thesis, PSU, December 2003) from trees at The American Chestnut Foundation's Glenn C. Price Research Farm in Meadowview, Virginia. Samples were taken from representative American and Chinese chestnut parents trees, their first-generation hybrids, and first, second, and third generation backcross hybrids (Table 1.).

Table 1. Populations sampled for morphology and dot-blot studies.

Population	Plantation and year planted[1]	Years since planting	Sample size for ISI study	Sample size for dot blots
American	Amer 2001	2	50	10
Chinese	CbyCs 2000	3	49	10
F_1	More F1s 1997	6	50	10
BC_1	JB1s 1999	4	60	30
BC_2	JB1s 1999	4	45	26
BC_3	Ilas 2000	3	49	28

[1]The plantation name refers to the chestnut plots at The American Chestnut Foundation's Glenn C. Price Research Farm in Meadowview, Virginia.

The population of American chestnuts represented the open-pollinated progeny of seven chestnuts growing wild in Smyth County, Virginia. The population of Chinese chestnuts was composed of two unique pedigrees, derived from controlled pollinations between two different sets of Chinese parents. All American chestnut parents in the backcross generations were the plantation-grown progeny of open-pollinated trees growing wild in the mountains of Virginia, except that one was itself a tree growing wild. Neither the American nor Chinese chestnut parents that were sampled were used as parent trees in any of the hybrid crosses. Twelve pedigrees of first-generation hybrids were sampled. These trees were the progeny of nine Chinese chestnut mother trees and 12 American chestnut father trees.

The populations of first-generation backcross trees sampled were progeny of a single American chestnut tree crossed with a single first-generation hybrid tree. Three pedigrees composed the population of second-generation backcross trees. The same first-generation backcross tree was used in each pedigree, but a different American chestnut parent was used in each cross. The population of third-generation backcross trees measured for this study comprised the progeny of a single second-generation backcross tree and a single American chestnut tree. There were no Chinese or American parents in common

between the first hybrid and any backcross generations or between the various backcross generations (see Hebard, this volume).

DNA Extraction, Digestion and Transfer

DNA was extracted from twig samples that were selected for DNA dot blot analysis from among 10 individuals among the chestnut parent and the first generation hybrid populations (Table 1). The samples were selected based on the Indices of Species Identity (ISI) determined by Diskin (2003) with the approximate ratio of 1:2. Thus, the selected samples from the 3 BC generations should have the same distribution and population coverage as the original set of twig samples used by Diskin. The sample sizes in the 3 BC generations used for DNA extraction were: 30 samples in BC1, 26 samples in BC2, and 28 samples in BC3.

DNA extraction followed the manufacturer's instructions (Qiagen DNAeasy kit). Methods for DNA restriction enzyme digestion, agarose gel electrophoresis and alkaline transfer of DNA to nylon membranes were as described by Sharp et al. (1988), with minor modifications such as the use of Hybond N+ membranes (Amersham). Total genomic DNA was digested to completion using HindIII restriction endonuclease (Gibco). The agarose gels were stained with ethidium bromide and only those gels in which all tracks of genomic DNA showed approximately equal amounts of DNA after UV photography were used for transfer.

DNA Quantification and DNA Dot Blot Preparation

The individual tree DNA samples were quantified with a GeneQuant (Amersham) spectrophotometer (A_{260}). All the DNA samples were diluted to 50ng/µL with ddH_2O. Methods for manual preparation of the DNA dot blots followed the protocol provided by the nylon membrane manufacturer (Amersham), except that 1 µL of 50ng/µL of denatured DNA sample was applied for each dot. In the simulation experiment, two repeated applications were applied to each dot, for a total of 100ng DNA. All the DNA samples were applied to the filters in a random order, following the random numbers generated by use of the MINITAB program (MINITAB 13.32, Minitab Inc. 2000). The applied ssDNA was fixed to the membranes using a UV crosslinker (Stratalinker®, Stratagene) for 30 sec.

Probe Labeling and Southern Hybridization

The labeling of probes with radioactive P-32, the hybridization methods and the detection of hybridization signals followed manufacturer's instructions (Amersham). Briefly, total genomic DNA was mechanically sheared by syringe, the length of probes was estimated by gel electrophoresis to be about 500bp. The probes were denatured by boiling for 5 min and then labeled with P-32 by following the random priming protocol (Invitrogen). The membrane was incubated at 65°C overnight in the prehybridization buffer with the denatured salmon sperm DNA.

For experiments involving genomic blocking DNA, DNA fragments of 100-200 bp length were obtained by autoclaving the total genomic DNA for 2 min. The required amount of blocking DNA, 1-10 µg mL^{-1}, was denatured by boiling for 10 min, added to the hybridization buffer surrounding the membrane and incubated at 65°C overnight. The labeled probe (10-20 ng mL^{-1}) was added and the incubation continued for 8-16 hr at 65°C in the hybridization incubator.

Washing and Signal Detection

After hybridization, weakly hybridized and unhybridized probe was removed by three washes of 30 min each in 1) 2 X SSC (20 X SSC: 3M sodium chloride, 0.3 M sodium citrate, pH7)/0.1% SDS (sodium dodecyl sulphate) at room temperature; 2) 0. 2 X SSC/0.1%SDS at 42°C; 3) 0.1XSSC/0.1%SDS at 65°C.

Hybridization sites were detected using a phosphor imager after the membranes had been exposed to the imaging screen for 2 h.

Signal Normalization and Quantification

Probe hybridization was measured quantitatively with a microcomputer-based image digitizing system TotalLab 2.00(Nonlinear Dynamics Ltd., 1996-2000). The intensity of the signals was digitized (Figure 3), and each measurement was normalized to the values of the positive controls (Figure 4). To compare the digitalized signal data from each dot, normalization was used to equalize the volumes in the dot images. This was accomplished by setting the normalized volume of dots from a serial dilution to specific values (positive controls) and then recalculating all other volumes relative to those values.

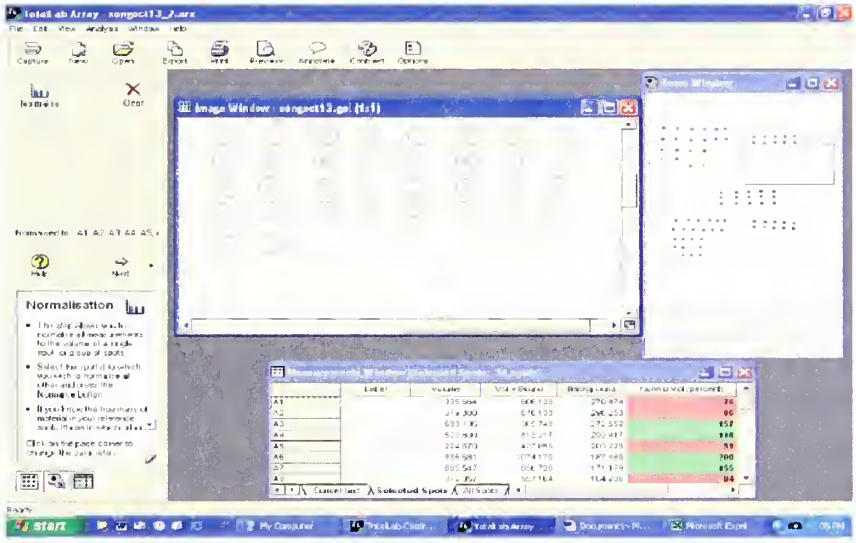

Figure 3. Screen capture of example signal quantification of dot blot using TotalLab 2.00.

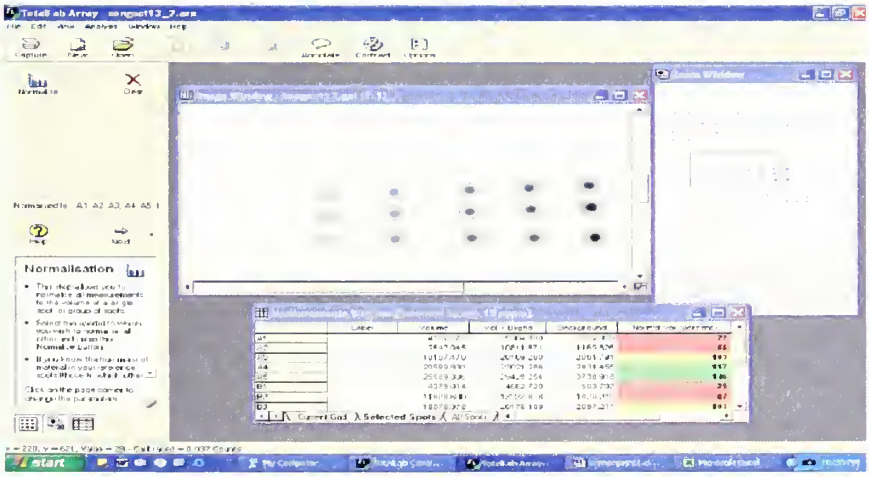

Figure 4. Screen capture of signal normalization by TotalLab 2.00 for positive control dilution example.

Statistical Analysis

Statistical analysis of the normalized dot blot signal data was performed using Minitab version 13.32 (Minitab Inc. 2000). Analysis of variance (ANOVA) was used to test the significance of mean differences within populations for signal intensity assuming equal variances. Brown and Forsythe's test was used to test for equal variance (Brown and Forsythe 1974). No data transformations were necessary.

Pearson's correlation coefficient was calculated to find the relationship between morphometric data and hybridization data (Minitab Inc. 2000).

RESULTS

Differentiation of American Chestnut and Chinese Chestnut

Preliminary experiments were conducted with parental DNAs to test the effectiveness of unlabeled American chestnut genomic DNA in blocking hybridization signal from shared sequences in the Chinese chestnut probe. The autoradiogram in Figure 5 shows the hybridization intensities obtained with labeled genomic Chinese chestnut probe hybridized to Southern blots of HindIII digests of parental and backcross generation genomic DNAs after two low stringencies washes. In the left panel of Figure 5, with no blocking DNA used, strong probe hybridization to DNA tracks from all generations is visible, and bands of restriction fragments from highly repeated DNA families are of similar intensity among samples. When the membrane was blocked with unlabelled DNA from American chestnut (right panel of Figure 5), the hybridization of Chinese chestnut DNA probe to the American and BC3 samples were greatly decreased, while the amount of hybridization to the Chinese, BC1 and BC2 samples were decreased to a lesser extent. In addition, the intensity of hybridization for the smallest band in Figure 5 (arrow) is increased in the F1 and BC samples after blocking, while the American sample maintains the same low intensity, suggesting that this restriction fragment is Chinese –specific and when the American genomic DNA was blocked, the band was more accessible to the probe.

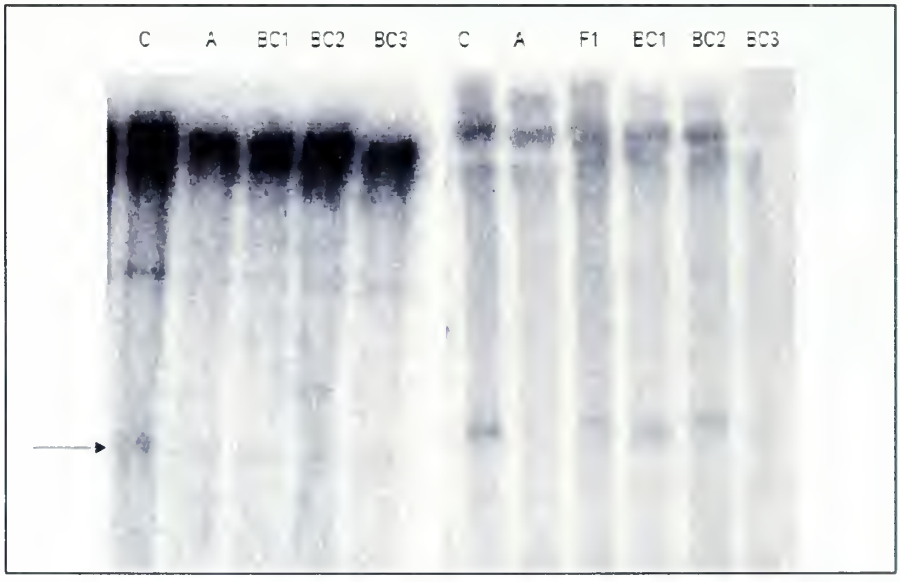

Figure 5. Southern Blot of genomic DNAs digested by HindIII, and hybridized against Chinese total DNA probe, labeled with P32. Left panel: No blocking DNA ; Right panel: American chestnut blocking DNA .

Signal Normalization With the Controls

In this project, we normalized signals within blots by using a serial dilution of known amounts of Chinese chestnut and American chestnut DNAs on the blots as positive controls (Figure 6). To avoid bias caused by experimental errors, the internal controls in each dot blot were used to normalize the dot signals among blots probed by Chinese total DNA, with American blocking DNA.

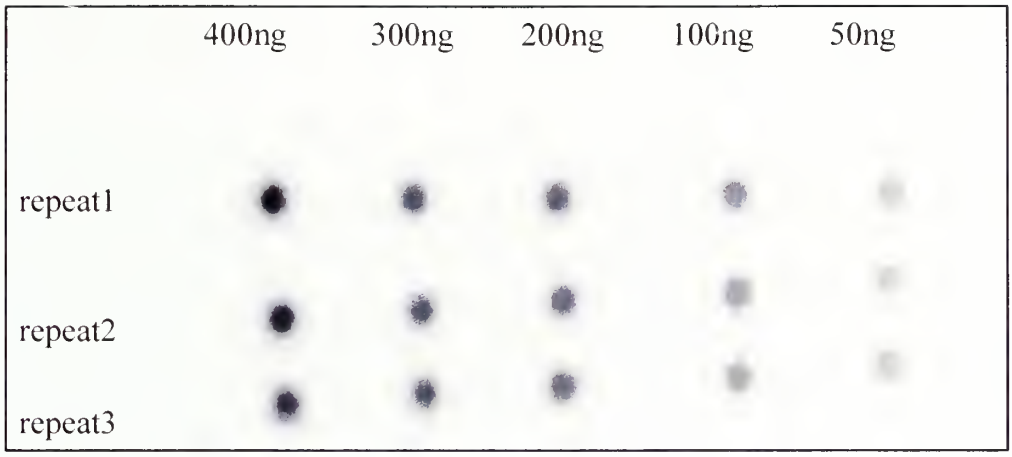

Figure 6. Autoradiogram of different amounts of Chinese DNA vs. Chinese total DNA probe, using American blocking DNA. This is used as a positive control to normalize dot signals.

Hybridization Variation Within and Between Chestnut Generations

A simulation experiment was conducted to test the level of sensitivity of the dot blot technique to genome variation among the chestnut generations. In the simulation experiment, mixtures of Chinese and American total DNAs equal to the average expected ratios for the F1 and 3 backcross generations (1:1 for F1; 1:3 for BC1; 1: 7 for BC2; 1:15 for BC3) were used to simulate average genome content in each generation. On the same blot, an equal amount of genomic DNA pooled from 5 individuals from each generation was applied and probed by Chinese total DNA with blocking DNA from American chestnut (Figure 7). As expected, the American chestnut DNA dot has the least signal intensity, while the Chinese DNA dot has the strongest signal. From F1 to BC3, the intensity of the dots decreased proportionately. When the amount of hybridization to the dots from the simulated DNA admixtures and the bulked DNAs were compared using TotalLab image analysis software, the results showed that the real and simulated mixtures had the same levels of intensity (Table 2), suggesting that on average, the backcross generations have the same ratio of Chinese chestnut genome and American chestnut genome as expected, which the dot-blot technique can faithfully detect.

To determine the extent of variation among individuals within and among backcross generations, a new blot was prepared with DNA dots from 10 individuals from BC1, 12 individuals from BC2 and 12 individuals from BC3, plus the parental DNAs as the internal controls. This blot was probed with labeled Chinese chestnut total DNA, blocked with unlabeled American Chestnut DNA (Figure 8). The signal intensity of each dot for this hybridization was measured in TotalLab (Table 3), and compared to the internal controls. The relative signal intensity, following TotalLab normalization, measured 328 for Chinese DNA and 19 for the American parental DNA. From the distribution of signal intensities among generations (Figure 9), we found, on average, that the BC1 individuals have stronger hybridization than BC2, while BC2 have stronger hybridization than BC3. This trend is as expected from the backcross program, i.e. that in general BC3 individuals have the least Chinese genome DNA remaining. However, much variation in hybridization was detected within each generation, opening the possibility for selection based on DNA content.

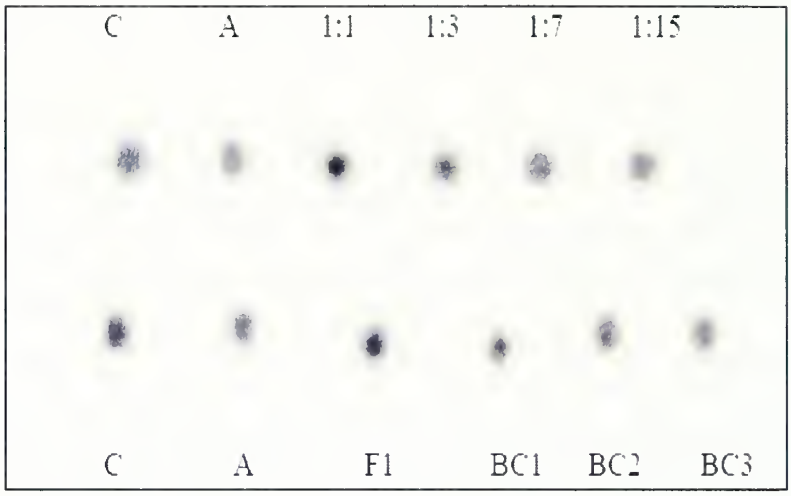

Figure 7. Autoradiogram of Dot Blot hybridization of mixtures of Chinese and American total DNAs vs. Chinese DNA probe, with blocking DNA from American chestnut. First Line: Mixtures of DNAs in the average expected ratios for F1 (1:1) and the 3 backcross generations (1:3 for BC1; 1: 7 for BC2; 1:15 for BC3). Second Line: Bulked DNA samples of six individuals from each generation. Each dot had 100 ng of genomic DNA delivered in 2uL. C, Chinese; A, American; F1, F1 generation; BC1, Backcross1 generation; BC2, Backcross2 generation; BC3, Backcross3 generation.

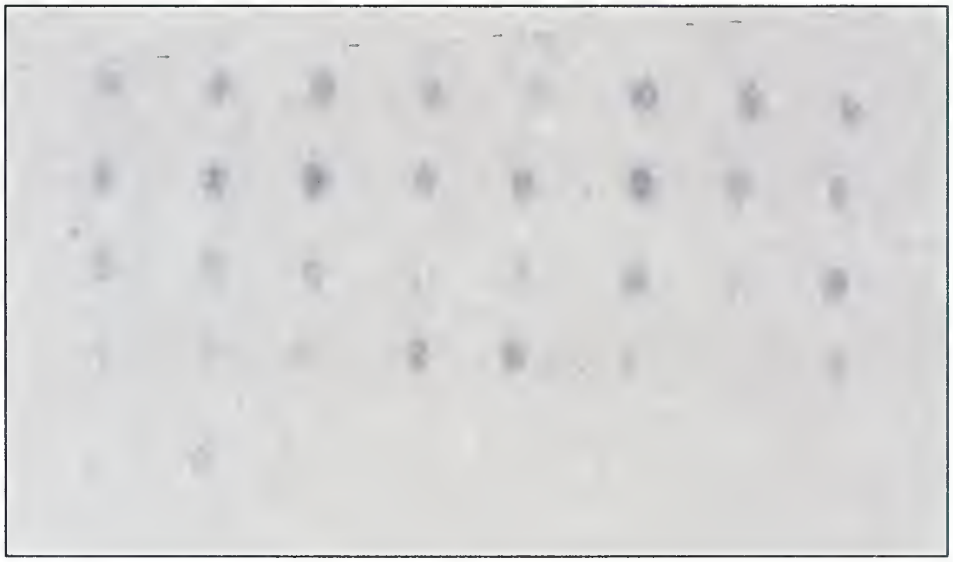

Figure 8. Autoradiogram of Dot Blot individual DNAs from BC1 (10 samples), BC2 (12 samples) and BC3 (12samples) vs. Chinese total DNA probe with blocking DNA from American. Each dot has 50 ng of genomic DNA delivered in 1 uL, all the samples are randomly arranged in this array.

To determine if the differences in hybridization intensities were statistically significant among the backcross generations, we used ANOVA in MINITAB to analyze the measurements by generations. For the result of one-way ANOVA (Figure 10), the P-value was 0.0000, showing that the variation of hybridization among the generations was highly significant. In the dotplot graph of the hybridization measurements (shown in Figure 11), the mean of the BC1 values was significantly greater than BC2, and BC2 was only slightly greater than BC3. For the ANOVA analysis, the variation within each generation was assumed to be equal. The statistical test for equal variation showed that the variation was indeed equal in each generation, although there were two individuals with greater variation than others in BC1, suggesting that there were some experimental errors or random errors in the procedure in those cases.

When we increased the sample size (Figure 13), however, the random errors were much smaller than in the experiment with smaller sample size.

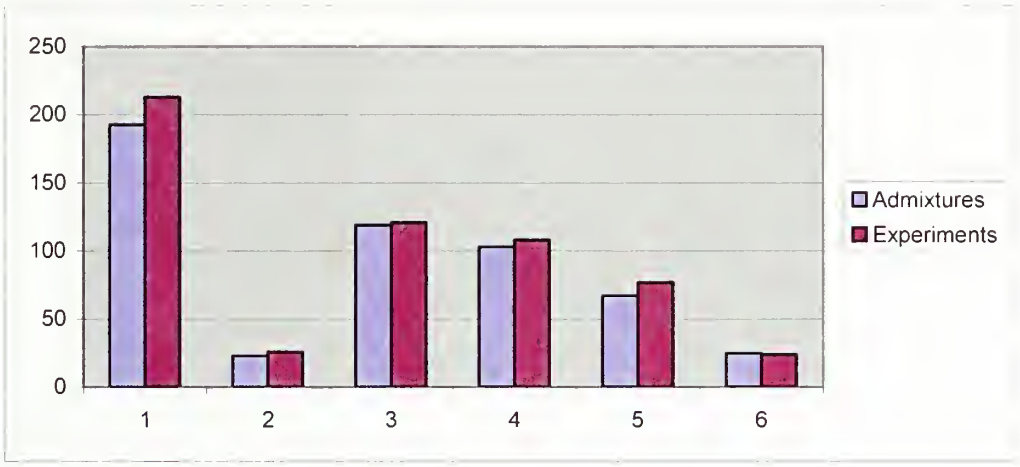

Table 2. Histogram of normalized data for admixtures and experimental samples in Figure 7.

generations / samples	BC1	BC2	BC3
1	156	94	70
2	121	110	38
3	192	79	33
4	142	104	31
5	148	118	39
6	294	81	32
7	140	119	28
8	286	52	18
9	163	111	57
10	159	65	36
11		68	92
12		79	47

Table 3. Values for normalized signal intensity data of hybridization shown in Figure 7 (Control values: 19 for American, 328 for Chinese DNA).

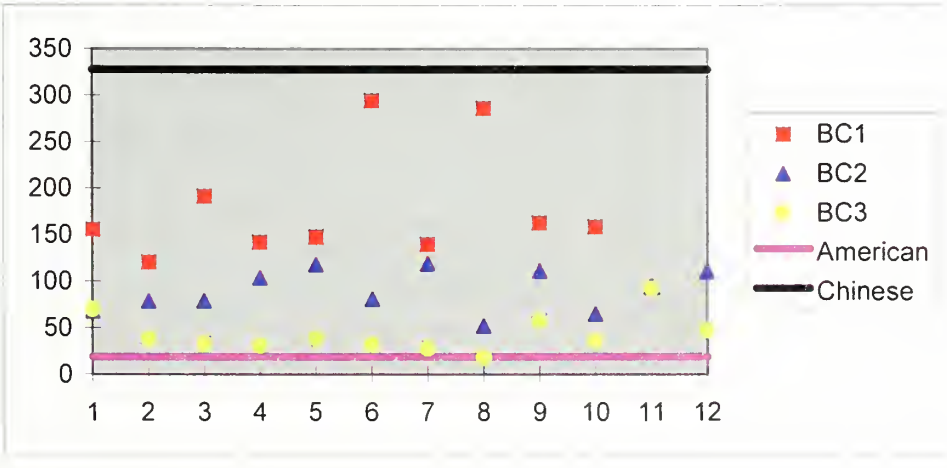

Figure 9. Signal discrimination for dot blot intensities between and within each BC generation, with American and Chinese parent as controls.

One-way ANOVA: nomalizations versus generation

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Analysis of Variance for nomaliza
Source      DF        SS        MS        F        P
generati     2    103780     51890    37.04    0.000
Error       31     43430      1401
Total       33    147210

                                      Individual 95% CIs For Mean
                                      Based on Pooled StDev
Level       N      Mean     StDev  ----+---------+---------+---------+----
1          10    130.10     60.76                                (----*----)
2          12     90.00     22.47            (---*---)
3          12     43.42     20.55  (----*---)
                                    ----+---------+---------+---------+----
Pooled StDev =    37.43             50        100       150       200
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Figure 10. One-way ANOVA test shows that differences in the signal intensity data between generations are significant.

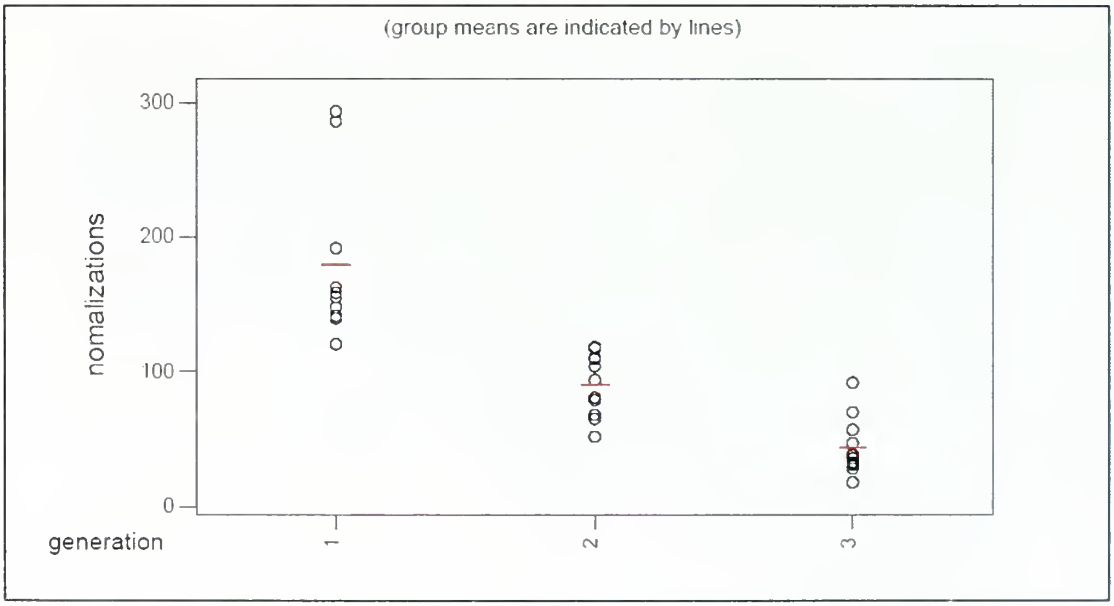

Figure 11. Dotplot of normalized dot blot data by BC generation. Red bars indicate mean values for each BC generation.

Relationship Between Variation in Hybridization Signal Intensities and Morphological Variation Among Backcross Generations

An inherent assumption with use of the dot blot protocol to screen for individuals with greater amount of American chestnut DNA within the backcross generations, was that a strong relationship should exist between variation in DNA at the genome level and the phenotypic, or the morphological, variation within BC generations. Diskin et al. (2006) (and previous chapter) measured twenty-four discriminating morphometric characteristics in each of the parental species, the first-generation hybrids, and the three backcross generations. Diskin used principal components analysis to develop an Index of Species

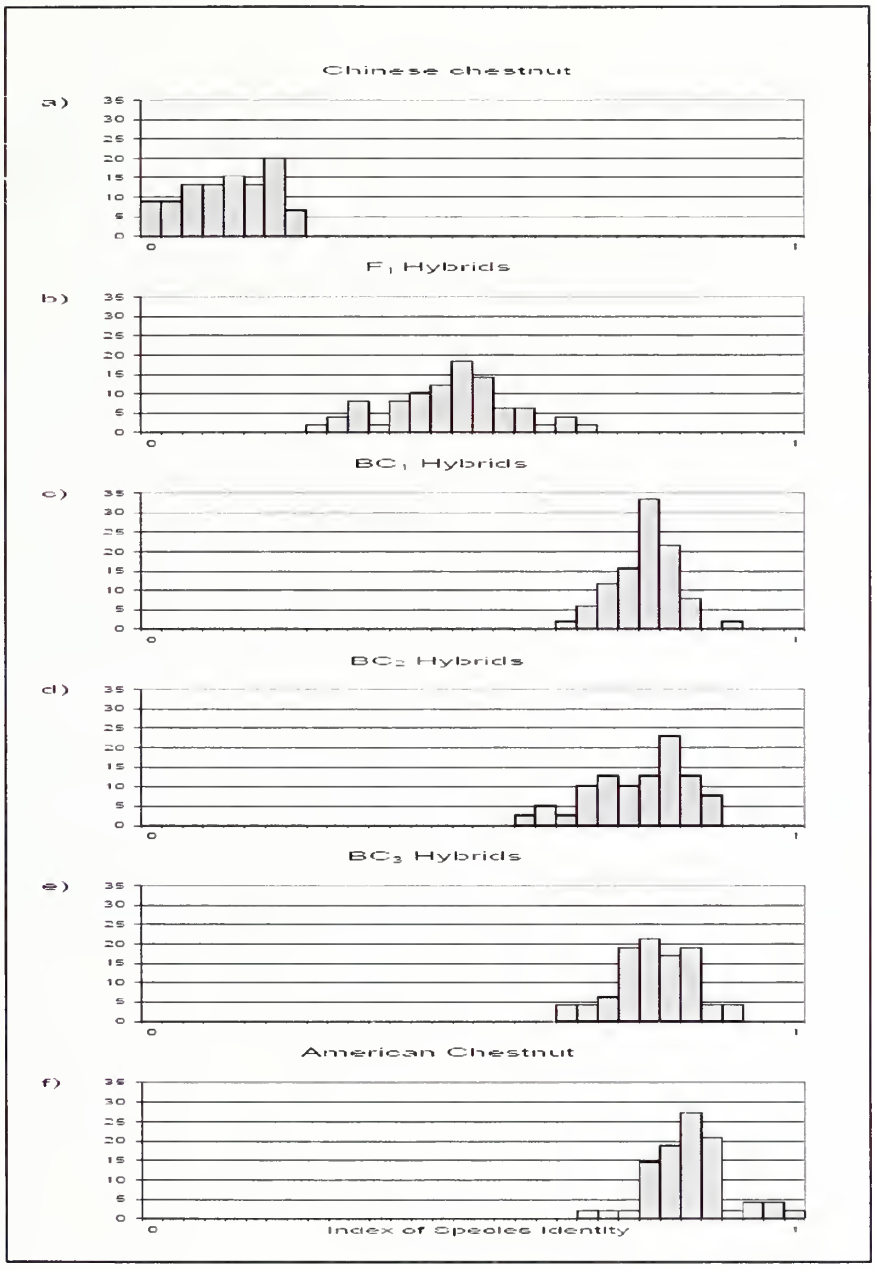

Figure 12. Index of Species Identity. Frequencies of the Index of Species Identity scores are plotted along the y-axis (from Diskin's thesis).

Identity (ISI) that described the aggregate morphology of the different populations relative to American and Chinese chestnut phenotypes. As expected, the morphologies of American and Chinese chestnut were at the extremes measured in his study. The first-generation hybrids were intermediate between the two parental species, and the three backcross generations had similar morphologies, distinct from Chinese chestnut and largely similar to American chestnut. American chestnut morphology was essentially recovered in the third backcross generation, based on ISI. To determine the relationship between variation in the genomic DNA hybridization data and the morphological variation, we prepared a DNA dot blot with 2 or 3 trees sampled from each bin of the frequencies of the ISI in each BC generation (Figure 12). The hybridization result is shown in Figure 13. All the samples were arranged in random on the blot, with three replications to decrease the hybridization bias and experimental error. The Pearson's correlation coefficient was calculated between the normalized signal intensity measurements and morphological ISI for each individual tree, yielding a value of -0.662, which is statistically significant (Figure 14).

Figure 13. Dot blot of selected samples (82 individuals with 8 controls) from the Diskin morphometric study (Complete random design with two replicates) probed with blocked, total Chinese DNA.

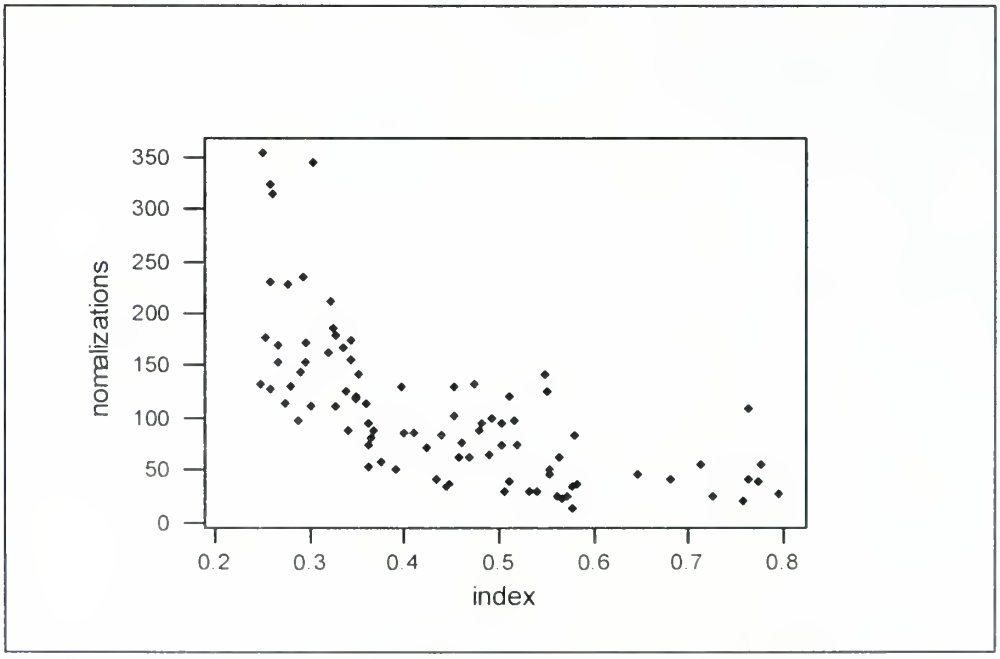

Figure 14. Plot of signal intensity data vs. morphological index data.
(Pearson correlation of normalizations and index = -0.662; P-Value = 0.000)

DISCUSSION AND CONCLUSIONS

Genome dot Hybridization Protocol

Genomic hybridization involves extraction of genomic DNA from one of the species of interest, for use as a probe by either Southern hybridization to DNA blots or by *in situ* hybridization to chromosome preparations from the species or hybrids being studied (Orgaard and Heslop-Harrison, 1994). Many of the DNA sequences within the two genomes under investigation may be sufficiently different so that genomic probing discriminates them. Those differences between species may include the different members of classes of repetitive DNA and species-specific DNAs. We do not know the detailed genomic differences between Chinese chestnut and American chestnut, but the results from the hybridization to DNA digests without the blocking DNA showed that their genomes have a high level of similarity, as expected for closely related species. The addition of an excess of unlabelled DNA from the American chestnut parents (blocking DNA) in our experiments substantially increased the specificity of the probe, enabling the two species to be distinguished by hybridization to DNA digests or dot blots. The effect of blocking in our experiments may be due to (a) hybridization between probe DNA (Chinese chestnut DNA) and common sequences in the blocking DNA (American chestnut DNA), (b) hybridization between the blocking DNA (American chestnut DNA) and common sequences on the membrane-immobilized DNA (Dot blot DNA) or (c) a combination of both.

The use of total genomic DNA, in combination with blocking, as a species-specific probe has several advantages. The use of genomic DNA as a probe avoids the need for the time-consuming and uncertain process of screening DNA clones from a library for clones that are specific to the American or Chinese chestnut genomes. Furthermore, it would not have been possible or practical to find enough American or Chinese specific sequences to cover those genomes in the present study. In contrast, the use of genomic probes is simple and straightforward in application, making it practical to develop a screening protocol for application within a large backcross breeding program.

Hybridization Variation Within and Among Backcross Generations

Because the backcrosses were made only to American parents, the Chinese chestnut genome was expected to be progressively diluted as backcrossing progressed. Statistically, it was expected that American genome should comprise on average half of the genome of individuals in the first interspecific hybrid, three-fourths of the genome of the first hybrid generation backcrossed to American, seven-eights of the genome of the first backcross generation backcrossed again to American, and fifteen-sixteenths of the third backcross population, if we assume the parents species have totally different genomes. Also, the variation of genome amounts should become smaller and smaller within each BC generation following successive selections for blight resistance and tree phenotypes. Correspondingly the difference in level of hybridization signal on dot blots should also be observed to decrease in magnitude between generations from the BC1 to BC3 generations ($1/4 \rightarrow 1/8 \rightarrow 1/16$).

Like Diskin's phenotypic ISI index, the DNA dot blot results with American and Chinese chestnut parental species trees in this study were distinct, and represent the two extreme cases, as shown by their scores in Figure 9. This is reasonable, and expected, as the study was based on the known genome differences between American and Chinese chestnut.

The hybridization signals of the populations measured in this study were summarized by their normalized data (Table 3). The progression towards American-like genome in each successive hybrid generation from BC1 to BC3 was apparent from the decrease in the means of the normalized signals (Figure 11). Also, as expected, the decrease in mean values from BC1 to BC2 was much greater than the decrease in values from BC2 to BC3. The decrease in magnitude of change towards the American chestnut genome value

among backcross generations fits expectations: each successive backcross generation is on average more American than the previous generations and the genome of the third backcross generation (mean = 43.42) approaches most closely that of American chestnut (normalization=19).

The ANOVA results showed that the mean differences among the BC generations were significant. This proved that the variation in dot blot hybridization is related to genomic variation, and not caused by experimental errors or random errors. The ANOVA test result of equal variance among each BC generation is not what we expected based on statistical considerations, however. The reasons for the equal variance in genome content among generations may be (1) that the sample size was not big enough to represent the whole population, bringing bias into the population sampling; or that (2) the genome differences between Chinese and American chestnut are actually too small to reliably distinguish the variances among the generations at the scale of dot blot sensitivity or that (3) additional variation is produced at each generation by recombination events during gamete formation.

Relationship Between the Hybridization Data and Morphology Data

To be able to screen for the individuals which are more American- like in the BC generations based on the results of dot blots, one should show that the variation in DNA content within generations is strongly related to the morphological variation. The ideal result would be a one to one relationship (Pearson's correlation coefficient=1 or -1).

In the project conducted by Matthew Diskin (Diskin 2003), an Index of Species Identity (ISI) was used to describe the aggregate morphology of the different populations. In our study prepared dot blots from samples selected from among those used by Diskin. The relationship between the dot blot hybridization data that we obtained and Diskin's ISI values was strong (Pearson's correlation coefficient = -0.662). A possible reason for this strong correlation could be that most of the genome sequences detected by the dot blot technique are expressed coding sequences that evolved at the same rate or along with the evolution of the morphological differences between the species. The negative value of the relationship is logical, because the ISI is positively related to American characteristics, while the hybridization data is negatively related to the amount of American genome DNA.

The relationship between morphological index and genomic dot blot signal intensities was not one to one, however, indicating that it is not possible to predict the morphometric differences between trees with 100% success based just on the differences in dot blot signals. Two possibilities could account for this. The first possible explanation arises from the fact that not all of the morphological variation that represents the species-specific characters were used to generate the ISI. If the morphological characteristics measured were not comprehensive enough, this could cause a bias in the ISI analysis. The genome-level variation assessed by dot blots should, in theory, be able to uncover differences in many more characteristics than is possible though phenotypic evaluation.

A second possible explanation for the differences between the ISI and dot blot results could be that the genomes of Chinese chestnut and American chestnut are highly similar because they are closely related. DNA sequences in the Chinese chestnut genomic probe that are highly similar to American chestnut sequences will be removed during the blocking step even though they may have very different expression patterns and cause different morphological characteristics. To minimize this concern, high stringencies were used in the dot blot filter washing steps and in probe blocking to ensure that only virtually identical sequences between American and Chinese chestnut species would be removed from the genomic probe.

In summary, this project has demonstrated that the dot blot technique can produce similar results to that obtained by the more painstaking and lengthy assessment of genotypes based on assessment of morphology for individuals in American chestnut backcross generations. The convenience, sensitivity

and rapidity of the dot blot approach should make the technique more suitable than phenotyping for screening large populations of trees and seedlings for American vs. Chinese genetic makeup. The observation that a significant amount of variation in dot blot signal intensity was observed among individuals in all three of backcross generations, indicates that the dot blot technique would be useful for selecting individuals with the greatest amount of American genome at each generation. The dot blot tool could thus greatly accelerate the goal of breeding blight resistant trees that have regained the genetic makeup of the American chestnut species.

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BIOLOGICAL DIMENSIONS OF THE GMO ISSUE

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Abstract: Genetic engineering is opening new opportunities in tree improvement, including access to novel genes for disease resistance. American chestnut is one of the tree species for which genetic transformation has been reported. Thus restoration of American chestnut could benefit from the genetic engineering technology, should suitable genes for blight resistance be identified. However, questions regarding the efficacy and safety of genetic engineering with trees, and other perennial plant species, have been raised including the stability of integration and expression of foreign genes, long term effects on non-target species, transgene dispersal through seed and pollen, etc. Many of these concerns could be alleviated if reliable approaches were available for the confinement of transgenes. The National Research Council assembled a committee of twelve researchers from various disciplines to evaluate the measures for biological confinement of transgenes. This paper will summarize the committee's findings regarding the unique concerns that arise in the application of GE to trees, and the potential that exists for applying bioconfinement methods with GE trees.

Keywords: National Academies; genetic engineering; trees; biological confinement; transgenes

INTRODUCTION

The ability to apply GE to plant improvement extends beyond annual crops to woody perennial plants including forest trees (reviewed by Pena and Seguin 2001) and fruit and nut trees (reviewed by Trifonova and Atanassov 1996) such as American chestnut (Conners et al, 2002a,b). Examples of successful GE with trees are appearing with increasing frequency in the scientific literature. Applications for field trials of GE trees have been submitted in the US, Canada, and the EU over the past 10 years for a wide variety of tree species ranging from pines to persimmons, and poplar to papaya (www.isb.vt.edu/cfdocs/fieldtests1.cfm).

The Committee on the Biological Confinement of Genetically Engineered Organisms was established by the National Research Council in 2002 to evaluate the status of and potential role of biological confinement technologies for Genetically Engineered Organisms (GEOs). The study was solicited by the U.S. Department of Agriculture. The National Research Council assembled a committee of 12 experts from various disciplines. The committee prepared its report over a 15 month period, meeting several times at the facilities of the National academies if Science in Washington DC and Irvine, California. In 2004, the Committee on the Biological Confinement of Genetically Engineered Organisms published its findings (Kirk, Carlson, et al 2004). This report is available in hardback edition from the National Academies Press, or as downloadable files at the NRC web site (http://www.nap.edu/books/0309090857/html/). This paper will review the background rationale for the study, the findings of the committee regarding trees and other woody perennials, and the committee's recommendations.

The federal policy framework for the regulation of biotechnology products was created in 1986. This framework followed a vigorous discussion amongst scientists and the public over a period of several years, and has continued to be debated since. Many new GEOs have been developed since the framework was established. More recently the application of GE technology has expanded from annual crops to

perennial plants and animals, including forest trees such as American chestnut. The expansion of GE to long lived organisms raised the issue amongst the regulatory community of how best to ensure confinement of transgenes over the long term (addressed in McLean and Charest 2000). Examples of failure of physical confinement protocols and the concerns raised in the public over the "escape" of transgenes into the food supply and the environment, suggested that it was time to also look at biological confinement opportunities.

The committee was charged with addressing the following questions:

1) What bioconfinement methods for GEOs are available, and how feasible, effective and costly are these methods?
2) What do we know about when and why methods fail, and what can be done to mitigate those failures?
3) When these methods are used in large-scale applications, what procedures can be used to detect and cull individuals for which the bioconfinement methods have failed?
4) What are the probable ecological consequences of large-scale use of bioconfinement methods at the population, community, and landscape levels?
5) What new data and knowledge are required for addressing these important questions?
6) What is the social acceptability of bioconfinement methods?

The committee focused on risks associated with the dispersal of a transgene or transgenic organism into a place, population, or biological community for which it was not intended. Long-lived species that disperse easily can present particular risks due to the inefficacy of physical confinement methods, and the potential for escapees to interact with wild populations. Thus the committee was asked to pay particular attention to transgenic fish, shellfish, trees, and grasses.

The concerns most often associated with risk of dispersal of transgenes include 1) The evolution of increased weediness or invasiveness; 2) Effects on nontarget populations — including humans; and 3) The potential for transgenes to disperse into the environment during field tests before being deregulated. The impact of transgene dispersal from perennial plants such as trees often involves issues of gene flow into natural populations. Gene flow issues with GEOs have been addressed in several studies, including trees (Slavov et al. 2002) from both theoretical and applied perspectives, and will not be dealt with much here.

WHEN WILL BIOCONFINEMENT BE NECESSARY FOR TREES?

GE trees are appearing with increasing frequency. Since 1989, more than 230 permits for field tests of GEO plants have been approved by the Animal and Plant Health Inspection Service in the United States, including 18 woody plant species. At least 65 permits have been granted in other countries for field trials on GE trees and other woody plants. The tree species for which field tests have been approved in include pines, persimmons, apples, walnuts, spruces, Sweetgum, aspen, plum, poplar, pear, and papaya (www.isb.vt.edu/cfdocs/fieldtests1.cfm).

Numerous traits are being engineered in trees, including lignin modification, increased growth and productivity, enhanced utilization of resources, pest and disease resistance, stress tolerance, herbicide resistance, optimization of mycorrhizal symbioses, phytoremediation of contaminated soils, and even production of anticancer drugs (Sederoff, 1999; Merkle and Dean 2000).

Specific concerns arise regarding the potential for escape of transgenes with forest trees. One concern is that long-distance gene movement occurs naturally with trees. Pollen and seeds from trees can be carried very long distances by wind, animal, and water vectors. Also, interfertile wild or feral relatives are quite

common among tree species. Since hybridization can occur so readily among trees, even exotic trees used for GE may require confinement if within pollen flow distance to related tree species. Another important issue is that trees are more likely to be keystone species within their ecosystems than other plants and animals. When keystone species are impacted, invasion and non-target impact consequences can be large and spread far beyond the species itself. Finally, forests trees carry a higher level of societal importance and impact than most agricultural species. Concerns over forest health are more than economic; they extend to issues of importance of place, esthetics, recreation, nature, and ecosystem services.

BIOCONFINEMENT TECHNOLOGIES

The committee searched the literature to identify as many bioconfinement methods as possible. Each technique or potential technique was described and its strengths and weaknesses evaluated. The following bioconfinement methods were reviewed:

Sterility
Mortality of Vegetative Propagules
Confining Pollen-Mediated Spread of Transgenes
Transgenes Absent from Seeds and Pollen
Artificially Induced Transgene Expression
Reducing Gene Flow to Crop Relatives
Repressible Seed Lethal Confinement
Cross-Incompatibility
Fitness Reduction in Transgenic Crop-Wild Progeny
Phenotypic and Fitness Handicaps
Reduced Exposure to Transgenic Traits

The category of sterility, included the use of interspecific hybrids, sterile triploids, unisexual plants lacking mates, transgenic sterility, ablation of reproductive organs, and reversible transgenic sterility (a "GURT" or Genetic Use Restriction Technology).

A GURT approach to bioconfinement of transgenes that has received a great deal of attention and research is known as "trait-genetic use restriction technology." Trait- GURTs are alternative approaches to reduce the effects caused by unwanted transgenes by activating a transgenic trait at a specific time through a specific artificial stimulus, such as a chemical spray. In this way, non-target organisms are spared long term exposure to the trait, the targeted species may be less likely to develop resistance, and GEOs that escape confinement should not have any selective advantage as the trait would not be expressed in the absence of the inducing agent in nature.

Several opportunities for confining pollen-mediated spread of transgenes were considered such as nontransgenic male sterility, transgenic male sterility, transgenes in chloroplast DNA transgenes in chloroplast DNA, and apomixis (for asexually produced seeds).

Creating GEOs in which the transgenes are absent from seeds and pollen has been proposed by use of non-transgenic scions on transgenic rootstock, and by programmed excision of transgenes before reproduction. Also, programmed cell death was evaluated as a means to induce mortality in vegetative propagules.

Approaches to reduce exposure of non-target organisms to transgenic traits through tissue-specific gene expression have also been proposed, including chloroplast-targeting of gene expression, limiting

expression to roots and tubers, vascular tissue-specific gene expression, flower- and fruit-specific gene expression, pollen-specific gene expression, and seed-specific gene expression.

EXAMPLES OF BIOCONFINEMENT METHODS

Sexual reproduction of genetically engineered plants can be blocked by including a gene that renders the organism either permanently sterile (nonreversible transgenic sterility) or conditionally sterile until an appropriate trigger is applied, such as the use of a chemical spray on a plant (reversible transgenic sterility).

Engineered sterility has its strengths and weaknesses for bioconfinement of GEOs. The main strength of the approach is in its overall effectiveness of confinement. Since with trees the primary risk of transgene escape is through pollen and seed, reliable sterility could overcome much of the risk associated with GE trees (Strauss, et al. 1995). However, there were weaknesses found in the general application of sterility as well, such as concerns that engineered sterility has not been adequately tested yet to determine how effective and reliable it will be for long-lived organisms, that it may be unsuitable for farmers who save seed from specific crops for replanting the next year, and it is still uncertain how well engineered sterility will be accepted by the public. With trees, the specific concern would be those cases in which the seed or fruit crop may be a highly desirable feature, such as in the production of mast for wildlife, in which cases engineered sterility would be counterproductive, unless reversible.

Although most of the above approaches are still untested in the field, their efficacy is generally recognized as having great potential. The options for engineering the nuclear genome to effect sterility in trees that are being tested in poplar includes ablation, gene suppression, and dominant negative mutants. Ablation of floral organs involves the regulated expression of a bacterial cytotoxin using a floral promoter from the species to be engineered. This approach targets expression of the cytotoxin to the tissues of the developing flower or floral buds, killing those tissues and thus preventing flowering. Engineering floral ablation in this manner is relatively easy to accomplish. However, very few if any genes are expressed absolutely exclusively in one cell type in plants. Promoters are more likely to be floral predominant rather than floral exclusive expression, thus raising the possibility that the cytotoxin will also be expressed in non-floral tissues. Thus with the ablation approach, it will be necessary to mitigate deleterious side effects in vegetative tissues prior to use. Another approach is to create dominant negative mutations in which a gene known to be essential for floral development is mutated in vitro to produce a protein that is stable in the cell but no longer active. Over-expressing that mutant gene produces an protein that interferes with the function of the wild type protein, causing sterility.

One of the most active areas of research in plants is gene suppression by RNA-interference (RNAi). RNAi is a natural system for regulation of gene expression in which small double-stranded RNA molecules interfere and thus suppress expression of endogenous genes. This can be engineered by expressing an inverted repeat of a small fragment of DNA homologous to the target gene (De Buck et al. 2001; Klahre et al, 2002). It should be possible to create sterile trees by RNAi targeted at genes that are of essential for floral development. Much is being learned about the genes regulating reproduction in trees, and this knowledge will offer opportunities for engineering sterility by manipulating the expression of nuclear genes as described above. Dr. Steven Strauss of The Tree Genomics, Biotechnology, and Breeding Program in the Department of Forest Science at Oregon State University, is conducting research on engineering sterility in GE poplar trees using floral genes in poplar as their model system. Table 1 lists some of the genes that are known to be involved in floral development in Populus species (as of May, 2004)

Table 1. Genes controlling flower development in Populus.

Arabidopsis Gene	Function in Arabidopsis	Poplar Homolog(s)
AGAMOUS (AG) *	Stamen & carpel identity	*PTAG1* *PTAG2*
APETALA3 (AP3) *	Petal & stamen identity	*PTD*
APETALA1 (AP1) *	Flower initiation; perianth identity	*PTAP1-1* *PTAP1-2*
LEAFY (LFY)	Flower initiation	*PTLF*

*MADS-box gene, member of a large plant gene family that regulates the expression (transcription) of other genes.

Field trials will be an important step in evaluating new GEO trees and in validating bioconfinement techniques such as engineered sterility. Strauss (2003) points out that a key issue in evaluating the safety of new GEOs and also of bioconfinement techniques should be the expected fitness consequences of escapes. That is, how great a risk to the environment or to people is posed by individual escapes, when bioconfinement techniques are not 100% effective. To be a risk, in most cases a transgene must amplify a great deal to have significant environmental impact. An individual GEO tree that has escaped into a natural population of millions of trees will have little of no impact on the overall makeup of that population, unless the transgene increases in frequency quickly. Small releases of GEOs thus have a major built-in safety buffer of scale in field trials. Unless the transgene provides a great differential benefit under strong selection pressure, the key force governing spread of the transgene will be genetic drift. Under genetic drift, a selectively neutral transgene is as likely to be lost from a natural population as maintained, and if not eliminated would require many generations to reach a frequency high enough to be a stable component of a natural population. Furthermore the maximum frequency that a neutral transgene might reach in a natural population will be lower, the fewer escapes from sterility that occur in the GEO population. This indicates that there should be a relatively high tolerance for incomplete sterility in field trials of GEO trees. If so, it may not be necessary to always accomplish 100% sterility to have an effective means of bioconfinement for transgenes.

APPLICATION OF GENETIC ENGINEERING TO AMERICAN CHESTNUT

The application of GE to American chestnut would involve transgenes for resistance to the blight resistance. A major issue with the release of GEOs with transgenes for resistance to disease and other pests is whether or not that will lead to resistance in the targeted organisms, as has occurred with the use of chemical pesticides. This is a particular concern with GEO trees which need to be able to rely on their transgenes for resistance over the course of many generations of the pest population. Several factors influence the development and containment of resistance in targeted pests. These factors include 1) the genetic basis of resistance, 2) the initial frequency of resistance alleles in the target pest population, 3) the competitiveness of resistant individuals in the pest populations, and 4) the resistance management strategy employed.

To ensure the long term usefulness of transgenes in GEOs for pest and disease resistance, it is necessary to have an effective plan to manage against the development of resistance to the transgene product in the pest populations. The key factors for a successful Resistance Management program include 1) knowledge of the biology and ecology of the pest, 2) low initial frequency of resistance genes in the pest populations, 3) low survival of pests when they are heterozygotic for the resistance genes, limiting transmission and build up of resistance genes in the pest population, and 4) the establishment of nearby refugia where the GEO is not present to ensure that susceptible alleles remain in high frequency in the pest populations.

Many different designs for refugia have been tested, but it most commonly it is recommended that 20% of the total area under cultivation, or crops, be planted as a refuge with non-GEO plants. The exact position of the refugia vary with the pest and crop species in question, but refugia may be embedded as rows within in the GEO field, or as borders or Blocks in the GEO field or even as a separate neighboring field. An alternate design includes a separate 20% Refuge that is sprayed with pesticide not related to the transgene product, to control the numbers of pests entering the GEO plots.

Figure 1. American chestnut tree with cankered trunk and new sprouts from the base that are not yet showing infection.

It is important to note, that there should be much less concern about the break down of resistance in chestnut trees than would normally be the case with crop plants. The sprouts that continue to arise from the roots of old wild chestnut trees will provide a large, continuously distributed natural refugia for the production of wild type blight spores (figure 1). The widespread presence wild-type sprouts that occurs throughout the natural range of American chestnut will provide the ideal refugia for preventing mutations in the *Cryphonectria* fungal populations from getting the upper hand. Thus, blight resistance in chestnut, whether from the back-cross breeding program or from genetic engineering, should be relatively stable over time in the forest, and perhaps require less active management than disease resistance in annual crops rather than more.

RECOMMENDATIONS OF THE COMMITTEE

After an exhaustive search which included interviews with experts from academia and the biotechnology industry, the committee reported the following major findings of its study:

- Most GEOs will not require confinement.
- The need for bioconfinement should be evaluated on a case-by-case basis.
- The use of redundant bioconfinement methods will be necessary in some cases.
- Biological confinement of GEOs should be undertaken in the context of an integrated confinement system.
- The need for confinement should be considered at the beginning of the design of a GEO, and be part of the entire development process, not just at the end.

The Committee on Biological Confinement of Genetically Engineered Organisms concluded its study with the following list of recommendations for the USDA.

- The need for bioconfinement should be determined on a case-by-case basis.
- The need for bioconfinement should be considered early in development of a GEO.
- The level of confinement needed should be defined early in development of GEO.
- The stringency of the integrated confinement system should reflect the predicted risk and severity of consequences of GEO escape.
- Bioconfinement techniques should be relevant to the temporal and spatial scales of field release.
- Confinement techniques should be tested experimentally.

- The phenotypes of novel GEOs should be compared with the progenitor organisms.
- Due to the long times required, field tests of bioconfinement methods with trees should be started as soon as possible, even if tests must first be conducted with GE trees not requiring confinement, to produce data the needed for later releases.
- Redundancy of methods can be used to improve confinement in high risk cases.
- An Integrated Confinement System should be used, involving technical, organizational, and regulatory elements.
- Methods should be developed to facilitate environmental monitoring for escapes, including easily identifiable markers, and sampling strategies.
- Transparency and public participation should be important components in developing and implementing appropriate bioconfinement approaches.
- The possibility of human error should be taken into account as a factor when determining bioconfinement methods and evaluating their efficacy.
- The international effects of failures of confinement should always be considered.
- International cooperation on the confinement of GEOs should be pursued.
- More research is required on methods for biological confinement of GEOs.

The committee went into some detail on the reasons why more scientific research is required. Research is needed to characterize ecological risks and consequences and develop methods and protocols for assessing the environment effects of confinement failure. Research is needed to develop reliable, safe, and environmentally sound bioconfinement methods. Research is needed to identify and develop methods and protocols to assess the efficacy of bioconfinement. Research is needed to identify economic, legal, ethical, and social factors that might influence the application of techniques, and their regulation. Research is needed to develop a better understanding of the dispersal biology of organisms targeted for genetic engineering and release. Research is needed to develop a better understanding of how species become invasive. Finally more research on risk assessment and safety management specific to GE trees (as a follow up to Lu et al 1999) are needed.

In addition, the Committee prepared a template for risk evaluation to be used in the process of planning GEOs, including in decision making on the need for and methods of biological confinement. This risk evaluation template could be a good tool for the National Park Service to use in evaluating the use of specific GE trees in ecosystem restoration and enhancement projects.

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NATIONAL PARK SERVICE POLICIES: HIGHLIGHTS FROM A WORKSHOP ON GENETICALLY MODIFIED ORGANISMS (GMOs) IN PARK LANDS

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Abstract: A workshop was sponsored by the National Park Service (NPS) to begin developing policy regarding use of genetically modified organisms (GMOs) within NPS resource management programs. GMOs were defined as organisms that contain gene combinations that do not occur naturally and were not created through traditional breeding practices. There are no current NPS policies that specifically prohibit use of GMOs on park lands. Therefore, for the purpose of the Chestnut Workshop, all technologies will be considered as potential tools to help restore American chestnuts to lands where they once occurred.

Key words: GMO / Workshop / NPS / Policy

INTRODUCTION

In April 2004, the National Park Service (NPS) sponsored a workshop to begin the process of developing policy regarding use of genetically modified organisms (GMOs) within NPS managed lands. The objectives of the workshop were to educate a core group of NPS personnel on various GMO issues, discuss where GMOs are currently being used on NPS lands, and identify unclear or controversial issues that would need to be resolved before policy development.

The GMO Workshop was structured much the same as the Chestnut Workshop, with two days of invited talks followed by a day of deliberations by NPS staff to begin drafting the major elements of a GMO policy. Invited talks covered basic concepts of GMO technology, current use of GMO products in North America, GMO products in development, current and potential use of GMOs in National Parks, potential environmental and social risks associated with GMOs, and a review of NPS policies that relate to GMO use in resource management programs. The Chestnut Workshop was scheduled to occur after the GMO Workshop in case policy described or developed at the GMO Workshop would clearly preclude use of genetically engineered products in an NPS chestnut restoration program.

WORKSHOP OUTCOMES

NPS policy is primarily contained within the publication *Management Policies* last revised in 2001. Policy can be supplemented or amended between revisions through formal issuance of a Directors Order. *Management Policies 2001* contains only brief direct references to GMOs, but does contain substantial guidelines on use of biological products to attain resource management goals. Those include: 1) guidance to restore extirpated native species using the closest available genetic material (4.4.2.2); 2) ability to introduce an exotic species in rare situations to meet specific management objectives (4.4.4.1); 3) ability to use a hybrid, subspecies, or improved variety where the natural variety cannot survive human-altered environmental conditions (4.4.4.1), and; 4) the ability to use bioengineered products for exotic pest management (4.4.5.4). There are no specific prohibitions against using GMOs within National Parks.

Genetically engineered agricultural crops, primarily herbicide tolerant and insect resistant corn and soybeans, are currently used in several agricultural lease programs by military and historical parks that are mandated to preserve farmed fields within the historic landscape. These parks generally lease fields to local farmers through cooperative use programs. GMO crops are often preferred because they are believed to decrease total use of pesticides, enable use of less toxic herbicides, and reduce fuel and labor costs. In addition, the use of GMO soybeans is so prevalent in U.S. agriculture that it is often difficult for farmers to obtain non-GMO seed in the commercial market.

A second area where GMO products are currently used in NPS management programs is recombinant wildlife vaccines such as rabies vaccines for coyotes and raccoon, canine distemper vaccines for black-footed ferrets and fox, and equine West Nile Virus vaccine for horses and mules. It is possible that other GMOs are being introduced on NPS lands without parks being aware they contain engineered genes.

A proposed working definition of GMOs was those organisms that contain gene combinations or gene sequences that do not occur naturally and were not created through traditional breeding practices. This generally refers to technologies that remove a small number of genes from one or more donor organisms that are inserted into a receiving organism, often across taxonomic groups. For NPS policy purposes, this would not include hybrids produced through artificial breeding or products of GMOs that do not contain viable genetic material, such as killed vaccines. There was also discussion on the benefits and limitations of categorizing all GMOs as exotic species for policy purposes.

The general feeling among workshop participants was that existing NPS policy would allow introduction of a GMO into a park if it met clearly defined management objectives and all feasible and prudent measures were taken to minimize risk or harm to other natural and cultural resources. Other policy considerations discussed included: 1) a prohibition against using GMOs for purely aesthetic purposes; 2) a prohibition against using GMOs if doing so would jeopardize park objectives or pose risk to human health or safety; 3) a prohibition against growing GMO crops that produce pharmaceuticals; 4) stringent risk & benefit analysis before considering introduction of a GMO, including full NEPA compliance; 5) consideration of possible gene flow to areas outside the park, particularly if certified organic farms are nearby; 6) monitoring use of GMOs outside of park boundaries for possible impact to park resources; 7) approval of GMO use on a case-by-case basis, and; 8) annual reporting of all GMOs used or released on NPS managed lands.

CONCLUSIONS

The process of constructing NPS policy on use of GMOs in park resource management programs has just begun, and will be developed and formalized over the coming years. At present, there are no specific prohibitions against using a GMO in park programs. The general feeling of workshop participants was that a GMO might be deemed acceptable if it is the best available product to meet a specific management goal, is generally considered safe by the scientific community, is accepted by the public, and is approved through the NEPA process. Therefore, for the purposes of the Chestnut Workshop, any technology can be discussed for possible incorporation into an NPS restoration program, including genetically modified trees and fungal pathogens.

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PLANTING TRIALS OF AMERICAN CHESTNUT IN CENTRAL APPALACHIAN MOUNTAINS

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Abstract: Field planting methods for American chestnut were examined in three separate trials to develop guidelines for the anticipated establishment of blight-resistant hybrid American chestnut into Appalachian forests. A direct-seed tree shelter test examined the effect on height growth of using five-foot tall tree shelters, vented or unvented, and no tree shelter treatments. A containerized/nursery stock test examined the effect on height growth of two nursery stocks (1-1 and 1-0) and greenhouse raised containerized stock in two container sizes (40 and 10 cu. in.) where half of each of the containerized stocks were given a 2.5-foot tree shelter. The direct-seed tree shelter test and the containerized/nursery stock test were located adjacent to each other at two sites. In addition, a site evaluation test examined the suitability of planting American chestnut at seven forest sites that formerly supported chestnut. In the absence of deer browsing, 5-foot tree shelters, vented or unvented, and 2.5-foot tree shelters, had no significant advantage over unsheltered treatments for seedling height after three field seasons. However, tree shelters were absolutely critical where deer browsing was frequent. 1-0 nursery stock did not grow significantly better than older 1-1 stock beyond the first field season, indicating that the extra year in the nursery was not necessary. Container size had no significant effect on growth rate. Planted seedlings competed well when natural regeneration was reset to ground level mechanically. Height and survival were unacceptably low for successful regeneration in all site evaluation trials, probably because of our inexperience in direct-seeding this species and intentionally casual approach to controlling competition and access by deer.

Keywords: direct-seed / container stock / nursery stock / tree shelter / regeneration / browse / transplant / seedlings / reforestation / restoration

INTRODUCTION

Field planting methods for American chestnut were examined to develop guidelines for the anticipated establishment of blight-resistant hybrid American chestnut into Appalachian forests. Much of what is known about American chestnut silviculture and regeneration ecology is derived from observations and studies that were carried out before the blight and the advent of forestry research as we now know it. Prior to the blight (*Cryphonectria parasitica*), American chestnut was found on gentle to steep slopes in mixtures with pines, oaks, and other hardwoods. The species avoided limestone-derived soils or bottoms with wet, cold, or shallow soils (Buckhout 1896, Zon 1904). Much of the reproduction was of coppice origin as most nuts were consumed by wild animals, livestock, and man (Buckhout 1896, Zon 1904). Seedlings that were able to germinate grew rapidly and formed long, vigorous, and wide spreading root systems similar to oak (Toumey and Korstian 1931). Regeneration was likely favored by widespread clearcutting and wildfires as chestnut was not as shade tolerant compared with beech, maple, and other potential competitors (Toumey and Korstian 1931).

Based on the authors' experience with oak, a relative of chestnut, trials were established to examine planting methods previously used for oak with the assumption that the results would be similar. Three

trials were performed: 1) a direct-seed tree shelter test, 2) a containerized/nursery stock test, and 3) a site evaluation test.

STUDY ONE

Protection from white-tailed deer (*Odocoileus virginianus*) is essential to establishing healthy forest seedlings in many parts of central Appalachia. When valuable disease-resistant American chestnut genotypes become available as planting stock for restoration, extraordinary protection measures such as use of fencing or tree shelters will be warranted. Tree shelters are relatively effective in protecting seedlings from deer until the tree grows above the deer browse line. The shelters also purportedly provide favorable growing conditions by moderating environmental extremes. However, shelters reduce light intensity, physically limit display of leaves to sunlight, and can increase temperatures around the seedling. Tree shelters often induce seedlings to grow faster in height than in diameter, and the resulting trees are typically spindly and susceptible of falling over if not supported. Shelters can have warmer internal temperatures compared to ambient conditions, which can accelerate bud break in the spring or delay hardening-off in the fall rendering trees susceptible to frost damage or winter injury. To prevent this, Tree Pro (West Lafayette, Indiana), a manufacturer of tree shelters, produces a vented shelter designed to maintain cooler temperatures and thus allow the seedling to acclimate more normally in autumn.

In 1997, a planting of direct-seeded American chestnut was established at Stone Valley (SV), The Pennsylvania State University's Experimental Forest in Huntingdon County, Pennsylvania. The test was designed to measure the effect on height growth of vented and unvented, five-foot-tall tree shelters (Tree Pro) vs. no tree shelter. For tree shelter treatments, American chestnut seed (obtained from Philip Lunde, Galesville, Wisconsin) was planted 1 inch below the soil surface and a vented or unvented tree shelter was erected over the planting spot. Shelters were pressed into the soil 1 in. Seeds for the unsheltered treatment were planted only within a seed protector, a 6 in. long piece of pre-split 1 in. diameter PVC pipe inserted into the ground to inhibit seed predation by small mammals. Fifty American chestnut seeds were planted for each treatment in a randomized complete block design. The study was established in a recent shelterwood harvest area (50 percent basal area removed) and a six-strand electric fence was erected around the entire site to provide protection from deer. Fortunately, this area has a very low deer population, and the need for protection from deer was minimal. Nonetheless, fencing allowed for a comparison of the shelters' effects on chestnut seedling growth without potential confounding from the effects of deer browsing. Native chestnut sprouts occur immediately next to the study area, so the site may be considered suitable for this species.

As anticipated, seedlings were significantly taller ($P<0.05$) in shelters compared to those left unsheltered for the first two years, but there was no statistical difference in height between the types of shelter in any year. Trees with no shelter began to catch up during the third growing season, to the point that there was no significant difference between treatments, but their mean height was still lower by about one foot compared to the sheltered treatments. Over the next three years, unsheltered trees became substantially taller than sheltered seedlings, although not significantly so, by nearly one, two, and three feet for each respective year. Certainly had there been deer pressure at this site unsheltered trees would have had much more difficulty in getting established and growing beyond the deer-browse line. After seven years of growth, the average heights are 21.1, 17.7, and 15.1 feet for unsheltered, vented-shelter, and unvented-shelter trees, respectively, and the tallest tree is 29.2 feet (unsheltered). This confirms the excellent suitability of this site for chestnut growth, not to mention the rather startling potential of this species to grow well in forest plantations. Blight is beginning to appear in the plantation.

A replicate test was begun in 1998 at the SV site and at a site in Tuscarora State Forest (TSF), about 70 miles south in Perry County, Pennsylvania. However, bears continually ravaged the SV planting by

tearing apart the shelters, and data collection was discontinued in 2002 because the plantation was too disrupted to provide meaningful results. Fortunately, the bears concentrated their efforts in this replicate and did minimal damage to the adjacent 1997 trial. The TSF site differed primarily in the amount of sunlight reaching the trees, as this plantation was established in a clearcut (rather than a shelterwood). This site also had an electrified fence, but the fence was largely ineffective and there was very heavy deer browsing on vegetation within the fence.

As in the 1997 SV test, initial heights at TSF were greater with tree shelters than without, and there was no significant difference in mean height between shelter types until after the fifth growing season when the trees had grown well above the tops of the shelters and trees in vented shelters were taller. In sharp contrast to the SV test, trees without shelters at TSF have never grown past competing vegetation or above the height of deer because of continual deer browsing. After six years of growth, the average heights are 2.2, 14.8, and 12.9 feet for unsheltered, vented-shelter, and unvented-shelter trees, respectively. The sheltered treatments are on par with parallel treatment means at SV of the same age.

The results of this study show that shelters do not benefit, and may even retard, the growth of American chestnut in forest plantations in the absence of deer pressure . Where deer were abundant, five-foot tree shelters were far more advantageous than no protection. No significant difference in growth was detected between trees in vented as opposed to unvented tree shelters.

STUDY TWO

While direct seeding will likely be the most efficient and cost-effective planting method for reestablishing chestnut into the central Appalachian forests, planting seedlings ensures higher survival rates per seed and permits greater control over final tree placement. Transplanted seedlings may also be competitively superior against the understory vegetation often encountered on forest sites.

Evaluations of the performance of containerized and nursery stock were performed at the SV and TSF sites adjacent to the previously mentioned direct-seeded, tree-shelter studies. The purpose of this study was to compare the growth of seedlings grown in two sizes of containers and raised in a greenhouse for three months (mid-February thru mid-May) and seedlings that had been grown under standard forest nursery conditions at a nearby Bureau of Forestry nursery for one or two years. The large and small containers measured 10 inches in length by 2.5 inches in diameter (40 cu. in.) and 8.25 inches in length by 1.5 inches in diameter (10 cu. in.), respectively. One hundred of each type of containerized stock was planted at each site, half of which also had a 2.5-foot shelter for limited deer protection. The nursery stock consisted of 20 each of 1-0 and 1-1 (one year in seedbed, one year in transplant bed) stock at each site. All seeds were obtained from the same source as the previous study. Each site was established in a completely randomized design in early- to mid-May of 1999. Existing vegetation was cleared to the ground with a brush cutter in an effort to reset competition. An auger with a 4-inch bit was used to plant the seedlings.

All planting stock transplanted well at both sites with better than 90 percent survival across all treatments and sites after two years in the field. More individuals began to die in the third year as other limiting factors (*e.g.*, deer pressure and competing vegetation) became increasingly severe.

At SV, heights of the 1-1 and 1-0 nursery-grown seedlings were significantly greater than those of younger, containerized seedlings after the first year in the field. There was no significant difference in height between 1-1 and 1-0 seedlings after two years in the field, which means that the extra year of growth in the nursery was not of practical advantage. The extra resources of the nursery-grown seedlings (initially larger root and shoot systems) were still providing a height advantage to those seedlings

compared to the younger, containerized material through five field seasons. The only exception was the large-container / no-shelter treatment, which was not significantly shorter than seedlings grown from 1-0 nursery stock after the fifth year; mean heights after five growing seasons were 9.4, 8.2, and 6.5 feet for 1-1, 1-0, and large-container / no-shelter treatments, respectively.

Containerized stock at SV was fairly consistent in height across treatments through three field seasons, but by the end of the fourth season, seedlings grown without shelters began to outgrow their sheltered counterparts. The height advantage of unsheltered seedlings was statistieally significant by age five. These results precisely mirror those of Study 1 with 5-foot tree shelters. After five years of growth, there was no significant height difference between seedlings started in different-sized containers and planted without shelters. but percentage survival was slightly higher with the seedlings that were started in the larger container (86 vs. 78 percent). In general, container-grown seedlings at SV have lagged in growth about a year behind the one-year-older 1-0 nursery stock, but not quite two years behind the two-years-older 1-1 stock.

At TSF the story was again vastly different due to significant deer pressure. While the nursery stock held height advantages over containerized material after the first growing season, 1-1 and 1-0 stock exhibited only meager growth in following years. Because all 1-1 and 1-0 seedlings were unprotected by shelters, the deer were able to continually browse new growth, and those seedling treatments have essentially failed at TSF. After five growing seasons, survival was only 40 and 45 percent for 1-0 and 1-1 nursery stock treatments, respectively.

Seedlings started in containers and provided with tree shelters have done better at TSF. Sheltered stock started in large containers have been taller than seedlings in other treatments since sometime in the second growing season, although sheltered seedlings started in small containers began to catch up by age five. However, with few exceptions, seedlings have never grown much beyond the height of the shelter itself (2.5 feet), and the mean height of all seedlings in shelters was only 3.3 feet after five field seasons. In addition to the deer pressure, these seedlings had to contend with an abundant cohort of yellow-poplar seedlings that became established simultaneously with the installation of this study. Yellow-poplar is a famously competitive species on good sites, but some of the chestnut seedlings that managed to escape the deer are competing fairly well with the yellow-poplar, typically just behind them in height. We will continue to watch these with great interest.

Overall, this study shows that transplanting of both nursery and greenhouse-grown, containerized stock can be accomplished with great success, but only if deer browsing is not a factor. In the absence of browsing, planted American chestnut seedlings can compete well with surrounding natural regeneration through five field seasons if that regeneration is reset mechanically at the time of field planting. If deer browsing is not a factor, seedlings grow better without tree shelters than with. There appears to be no advantage to using 1-1 nursery stock, which is costly to produce and difficult to plant compared to 1-0 nursery stock.

STUDY THREE

In our latest phase of experimental chestnut plantings, we are examining the suitability for American chestnut of a range of native and relatively undisturbed forest sites by attempting direct-seeded establishment of chestnut plantations. The Pennsylvania Chapter of the American Chestnut Foundation provided seed for this study. Seven sites were established with 50 seeds each in 2001 and this was replicated in 2002. The sites vary in soil type, elevation, aspect, competing ground vegetation, and undoubtedly other important respects. All plantations were established in feneed areas that had recently

been harvested, or in areas that were fenced to encourage regeneration where little vegetation existed due to over-browsing by deer.

Two of the sites were located approximately 80 miles south of State College, PA, in Tuscarora State Forest. "Eby Ridge" has a Hazleton extremely stony, sandy loam soil that consists of a deep, well-drained, strongly acid soil that formed under sandstone residuum. It sits on a south-facing slope at 1237 ft. It had little competing ground vegetation after the first year of establishment, but the 2001 trial later developed considerable hardwood competition. "Dead End Road" consists of the same soil type, but sits on the east side of a ridge top at 2026 ft. and has considerable competition with a thick carpet of *Vaccinium*. While this site is fenced, it still has a considerable presence of deer.

"Deep Hollow" is located approximately 40 miles east of State College in Bald Eagle State Forest. The soil type is delineated as Dystrochrepts bouldery great group, but it appears very similar in texture and stoniness to Hazleton. In fact, this site bears a very strong resemblance to Eby Ridge in most regards. Deep Hollow sits on a south-facing slope at 1302 ft. and had only a small amount of competing *Vaccinium* on the 2001 trial, but a greater density on the 2002 trial.

Four sites are located within Rothrock State Forest south of State College. "Galbraith Gap" has a Laidig extremely stony loam soil that consists of a deep, well-drained, strongly acid soil that formed under sandstone and siltstone alluvium. It sits on a south-facing slope at 1930 ft. and has a thick carpet of *Vaccinium*. Here again, although this is a fenced area, there is a considerable presence of deer. "Pine Swamp Road" has a Hazleton-DeKalb soil type very similar to the Hazleton series. It sits on an east-facing slope at 1442 ft. and has a thick carpet of hay-scented fern. "Owl Gap" has a Buchanan extremely stony loam soil that consists of a very deep, moderately well-drained, slowly permeable, very strongly acid soil. Buchanan series soils formed in colluvium on mountain footslopes, sideslopes, and in valleys that were weathered from acid sandstone, quartzite, siltstone, and shale. It sits on a north-facing slope at 1403 ft. and has a thick carpet of hay-scented fern. "Spruce Mountain" also has a Buchanan soil type. It sits on a south-facing slope at 1593 ft. and has some competition with grasses, forbs, and regenerating sassafras and red maple.

All sites were mechanically cleared before planting with a brush cutter to reset competing vegetation. Radicles had emerged from most seeds while in storage and were cut back to facilitate planting in seed protectors (described above) that were used for protection against small mammals. The competition described above was present at the time of data collection in late September 2001 and was similar through 2003.

2001 Trial

After three years in the field, seedlings at Owl Gap were tallest (20.0 inches) and had the best survival (66 percent) compared to the other sites. Spruce Mountain was second best in height (17.4 inches), but ranked last in survival (26 percent). Eby Ridge and Deep Hollow were close behind in height (15.4 and 15.0 inches, respectively), but had lower survival (38 and 47 percent, respectively). The rapid reemergence and density of the hay-scented fern at Pine Swamp Road seemed to be too much for the chestnut seedlings to develop much height (7.5 inches) while survival slowly declined to 46%. Finally, the intensity of deer browsing at Dead End Road and Galbraith Gap made the growth and survival of any edible plant almost impossible, so work at these two sites was discontinued. However, growth was unacceptably poor at *all* sites, and even the best survival rate (66 percent) is borderline by our standards of a successful forest plantation.

2002 Trial

Owl Gap, Eby Ridge, and Spruce Mountain had the tallest trees (20.0, 18.7, and 14.0 inches) at the end of the second year. Mean seedling height at Deep Hollow was substantially less (9.4 inches), and the seedlings under the dense hay-scented fern cover at Pine Swamp Road reached a mean height of only 5.5 inches. Mean heights after two years in the field in this trial were similar to mean heights after three years in the field in the 2001 trial, but no site is exhibiting the growth that chestnut is capable of. Survival at all sites was unacceptably poor, with Owl Gap having the highest at only 48 percent.

These trials are still in their infancy, and our methods need to be refined until we can get better survival and more acceptable early height growth. Other than deer browsing, competing vegetation appeared to be one of the greatest factors affecting height at all sites, and resetting it mechanically back to the ground level apparently did not give direct-seeded chestnut sufficient early advantage for successful establishment. These plantations would have been failures if the goal had been to restore American chestnut to these sites. Plans to continue these studies with additional trials utilizing chemical control of competition and planted seedlings are underway.

CONCLUSIONS

After examining several planting methods we still believe direct seeding will be the most efficient and economical method of reestablishing American chestnut when large numbers of seed become available, if competition and deer browsing can be adequately controlled or avoided, as these appear to be absolutely critical factors when planting chestnut from seed. The growth and survival of seedlings at SV in Study 1 demonstrate the remarkably vigorous potential of American chestnut in forest plantations, but the failures at TSF and in Study 3 show that this potential is fragile if conditions are not right. With a small amount of seed or available land area, one may well chose to use either bareroot or containerized nursery stock to ensure greater survival. We found 1-0 nursery stock to be just as good as 1-1, thus there is no real need for the extra year at the nursery. Containerized material had an excellent success rate but was costly to produce.

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PLANTING TRIALS WITH AMERICAN CHESTNUT IN SOUTHERN APPALACHIAN FORESTS (transcript of presentation)

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INTRODUCTION

We are really just getting started in our studies with American chestnut in the Forest Service. I don't have as many results to show as the folks at Penn State. I do first want to give some credit to the people who have really done most of the work in this study – particularly Chuck Rhodes, who was at the University of Kentucky at the time we started this and is now with the Forest Service out in Colorado and Jeff Lewis, who is a silviculturist in the Morgan Ranger District of the Daniel Boone National Forest.

I am going to start off with this profound statement, '*planted chestnut is only going to make it if it beats its competition.*' The name of the game in forest regeneration is beating the competition. There are a couple ways to beat the competition. One way, from a silvicultural standpoint, at least, is that you can control the competition. We have all been exposed to weed eaters and herbicides and probably other techniques we can use. I wonder if in some cases, at least on a broad scale, whether an approach that relies strictly on control of competition is logistically and economically feasible. It may be, it may not be, I don't know the answer to that. This may just be a question of how much value we put into restoring chestnut. There may be other ways, other approaches, that we can take that might allow us to use less competition control and perhaps avoid some of the difficulties and expense with trying to reintroduce American chestnut on say a 100,000-acre piece of ground.

WHAT DO WE KNOW ABOUT CHESTNUT?

Well, we know something about its distribution. I was asked to talk specifically about the southern Appalachians. We know about where chestnut grew, and we also know that a variety of species now occupy the space once occupied by chestnut. But its function has probably not been entirely replaced in these ecosystems in the southern Appalachians, or elsewhere. Why haven't those functions been replaced? It was the most numerous, most abundant tree in the southern Appalachians. As an individual species, it was the most numerous. It was an important food source for many animals. One thing learned recently is that it was extremely important as long-term durable coarse woody debris in both terrestrial and aquatic systems. That's something that's disappearing now, and I think some people are concerned that we don't have a good replacement. And of course it was commercially valuable and had great utility. So these are some of the reasons why we want to regenerate chestnut and restore it.

We also know that chestnut was a large tree. We know that it grew over a broad range of sites and soils. It certainly grew on mesic sites, where it reached its greatest development, but it also grew and was actually more dominant on sub-xeric to even xeric sites. It grew over a broad elevational range, as well, in the southern Appalachians. We know that its wood was durable and extensively used. It regenerates well from stool shoots and from seeds, which are borne regularly in abundance. The rate of growth is very rapid, being greater than any other hardwood in the region.

Unfortunately, as has been already pointed out at this meeting, by the time we began to get systematic scientific investigations going in the eastern United States, the chestnut was on its way out. Figure 1, as an example, is a picture taken in 1905. This photo was taken on Bent Creek, and this guy was studying what was replacing chestnut. This picture was taken probably just a few hundred yards from the North Carolina Arboretum. We really never did learn much about the silvical or ecological characteristics of American chestnut before its decline.

Figure 1. Early study on American chestnut regeneration, Bent Creek, 1905.

WHAT DO WE NEED TO KNOW?

The work of Ayers and Ashe (1905) restates what we discussed earlier – chestnut grew very rapidly. What they observed as they traversed the woods is that yellow-poplar also grew very rapidly. And I particularly like their description of yellow-poplar and chestnut 'freely seeding' into openings created after cutting (Figure 2). The young poplars often vastly outnumbered the chestnuts, but the chestnut grew faster and overtopped the poplar and suppressed it, which is an interesting observation.

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The more rapid height growth of the chestnut in large measure accounts for the scant reproduction of the yellow poplar in culled woods, for poplar and chestnut both freely seed such openings, the young poplars often outnumbering

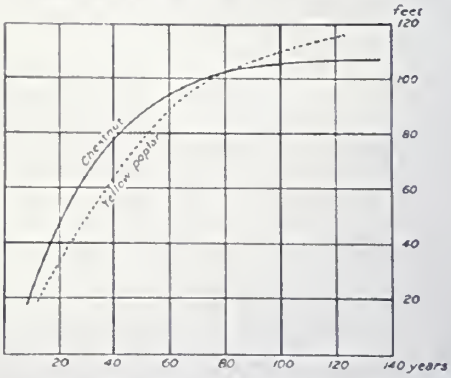

FIG. 2.—Curves showing the rates of height growth of yellow poplar and chestnut on good soil.

the chestnuts, but the chestnut grows far more rapidly, overtops the poplar, and suppresses it.

Figure 2. Yellow-poplar and American chestnut competition (Ayers and Ashe 1905).

But Ayers and Ashe were not the only people around during the period before chestnut began to decline. Another very keen observer was E. H. Frothingham. He came through the Asheville area in the early part of the century, actually about 1914-1915, as the national forests were being set up pursuant to the Weeks Act. Frothingham also later became the first director of what is now the Southern Research Station. He was also Director of the Appalachian Forest Experiment Station for the U.S. Forest Service from 1921 to 1934. He did a report (Frothingham 1917) on the cut-over areas of the southern Appalachians, from North Carolina to West Virginia. It was a very complete report. It was a thorough observational study, looking at many different cuts and what was becoming reestablished in those cuts.

But while Ayers and Ashe seemed to suggest that new seedlings were a viable source of regeneration in forest openings, that was not what Frothingham routinely observed. In fact, in only one case in all of his observations did he indicate that he thought that the source of successful regeneration was coming from seedlings. It was almost always from sprouts. There are some contemporary scientists (e.g., Billo, Paillet, McNab) who have painted a similar picture of the natural regeneration ecology of chestnut, indicating that it might not freely regenerate from new seedlings, at least over all sites.

Frothingham (1917) also stated that: "Observational studies of cut-over areas have the great disadvantage of missing the most important stages in the reproduction on cut-over areas, and especially the conditions prevailing immediately before and after the year of cutting."

This was a criticism he made of his own study and other observational studies. In observational studies you come in after the fact and maybe look at a time series of different cuts on different sites. This approach misses some of the most important information about factors that ultimately influence species composition. And over the last 40 years we have come to realize that one of the most important things that we need to know about species is their characteristic regeneration strategy – what is the source of successful reproduction? Reproduction comes from a finite number of sources: from new seedlings established after disturbance, from advance reproduction that persists through disturbance, and from sprouts from stumps or roots that also persist through disturbance.

WHAT DO WE KNOW ABOUT CHESTNUT REGENERATION?

We really don't know for sure where successful natural regeneration of American chestnut came from. Although we know that sprouts were successful, we don't necessarily know that seedlings were often successful. Can chestnut become established after disturbance and grow rapidly enough to compete successfully or does it have to persist through disturbance as advance reproduction, like the oaks and hickories and most other species that we have in the southern Appalachians? Perhaps it is like the oaks, which don't grow rapidly from small advanced reproduction as black cherry does, just as an example. Like the oaks, it might require a larger root system to sustain rapid height growth and to compete successfully. If it does require a large root system, what kind of light regime is needed to create or develop that large root system, and how long does it take for it to develop under those conditions?

I think the last question is extremely important. Does it behave consistently; is its regeneration strategy the same everywhere? Is it the same on xeric sites as it is on mesic sites? Is regeneration the same in Massachusetts as it is in the southern Appalachians? I don't think we really know the answers to these questions. It could well be that we'll find multiple strategies necessary in order to regenerate chestnut.

PRELIMINARY FINDINGS

In our initial study, which is only a couple of years old now, we decided to look at regeneration as well as we could with the limited plant material that we had. We are looking at the most fundamental of these questions, I think, which is where does successful regeneration come from if we are going to plant it? So we decided we would plant American chestnut seedlings in a very open situation, in a clearcut or a very low residual basal area oak shelterwood or that we would plant under an oak canopy and treat it in such a way that we would have a modest increase in light to be released several years later by an overstory removal. Those were the two basic treatments (open setting vs. under a canopy), and again, we were limited by the amount of chestnut plant material we had. The other thing we wanted to do in this study was to compare these strategies, planting in the open versus planting under a modified stand structure. But also we planted on both moist sites where yellow-poplar is a competitor and on drier sites where it is

not. As Phelps pointed out, yellow-poplar can be a fairly severe competitor. In the Southern Appalachians, it's maybe a little different than in Pennsylvania. But that is the nature of the study. At this point, all of the plantings are in eastern Kentucky, two of them are on National forest land, one at a college, one on a state forest, and the other on a school forest.

Figure 3. Open shelterwood planting site (20 ft^2 residual basal area).

We are in the process of completing the necessary paperwork to actually put a study on the Bent Creek Experimental Forest. It's been a long time coming, but we'll put in that study in the next year or two. But in the current location of the study the treatment was a very open stand condition, in this case a low residual basal area shelterwood (Figure 3). We chose this particular treatment because this is consistent with the forest plan on National Forest Land. We would not necessarily have been allowed to clearcut on National Forest Land. We created very open conditions with this shelterwood cut, creating an open shelterwood with 20 ft^2 of residual basal area. This was also a midstory removal, where we took out perhaps 20%-30% of the basal area from below, using herbicide injections for that. Again we did those treatments on some north facing slopes, mesic sites, where yellow-poplar was a competitor, as well as on south-facing slopes, where yellow-poplar was not a competitor.

In summary, we have an open shelterwood cut on a xeric site (Figure 4), an open shelterwood cut on a mesic site (Figure 5), a midstory removal on a xeric site (Figure 6), and a midstory removal on a mesic site (Figure 7).

Figure 4. Open shelterwood cut on xeric site.

Figure 5. Open shelterwood cut on mesic site.

Figure 6. Midstory removal on xeric site.

Figure 7. Midstory removal on mesic site.

EARLY CONCLUSIONS

So far we don't see any differences in survival among the sites and treatments (Figure 8). Chestnut planted in the open grows faster than those planted under more shaded conditions (Figure 9). But all of the seedlings have grown. It will be another 3-4 years probably before we are really able to definitely assess the competitive status of chestnut planted in the open.

This story is not complete yet. You can see the chestnut seedling in the open shelterwood site in Figure 5. But on the other hand, there is also yellow-poplar. We don't know whether that chestnut can actually compete with yellow-poplar, the primary competitor, without supplemental competition control. We certainly found that oaks cannot do so. So that is really the story we are looking at here, especially in the southern Appalachians – whether or not the planted chestnut can keep up with some of the competition, notably on the higher quality sites. And especially whether it can keep up with yellow-poplar on the really mesic sites. It's going to be quite awhile before we are able to assess the competitive status of chestnut that was planted under a modified canopy and then subsequently released. That is probably about a decade away.

So we do not know how much competition control is going to be necessary to establish chestnut under various conditions. But, I guess I do question from a logistical and economic point of view whether or not we can effectively reintroduce chestnut on a very, very large scale and control competition as intensively as may need to be done, particularly on mesic sites. It's entirely possible that we may end up looking at different planting strategies, one for one more xeric sites and a different strategy for mesic sites. I don't think we know this yet, and only time will tell, at least in the southern Appalachians.

Finally, I would also like to pose a question that might spur some additional research. If we find that we need to adopt multiple strategies or if we simply find that planting under a modified canopy might be a

viable strategy, do we need to look at how we produce seedlings that are well adapted to the environments in which they are being planted? This work should really operate in tandem. I am not sure that we are going to get perfect answers, as we are limited in terms of the plant material that we have to work with. But it could well be that we might want to modify the way that we produce seedlings if we are going to be planting them in more shaded environments.

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Ecosystem Restoration and Federal Land Policy:

Reexamination in Light of The American Chestnut Restoration Effort

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The suite of federal laws defining the goals and policies regarding ecosystem and biodiversity management on federal lands begin with the presumption in natural systems that "if it ain't broke, don't fix it." These laws presume that waiting and studying the system will (1) enable us to develop a comprehensive plan of action that will prevent or minimize future harm and (2) waiting will not cause further harm.

Beginning with the National Environmental Policy Act ("NEPA")[2] and continuing with the various laws defining the terms of ecosystem management on federal lands,[3] those laws have required strict scrutiny of new actions to assure that they do not disrupt existing conditions, which are deemed "natural." This presumption is reflected in the requirement under the NEPA regulations that every environmental impact statement include consideration of the "no-action" alternative.[4] Although the leading federal law directly aimed at biodiversity conservation, the Endangered Species Act,[5] recognizes the need for active intervention and management in calling for the development and implementation of recovery plans for threatened and endangered species, even that Act focuses heavily upon the prevention of human activities adversely affecting those species or their critical habitat. In fact, prohibitions on "taking" individuals can often prevent application of actions to restore habitat and support the species (Bean 2003). In many cases, if not the majority of cases, the underlying presumption in favor of leaving "Mother Nature" alone without a good showing that we will not unduly disturb her is a good approximation of reality, insofar as it has been human activities that have caused much of the environmental disruption that has been observed to date.

However, human influence has become so pervasive globally that the presumption against human activity is no longer valid in many cases. Human intervention is often necessary to maintain or restore damaged ecosystems and species populations. In these cases, human intervention is required to manage a "natural system" to preserve the system's existing characteristics or to restore the system (Flannery 2001). In these cases, the laws designed to prevent injury by requiring that the government wait and study before acting can also deter or prevent altogether ameliorative actions. For example, human introduction of pests and diseases can threaten species and disrupt ecosystems without human intervention. Introduction of the chestnut blight reduced a species which formerly was found throughout the hardwood forests of the eastern North America and comprised as much as 25 to 50% of the forest composition where it was dominant to a shrub growing from root sprouts (Oak 2002; Rhoades 2001; Russell 1987). Human introduction of the wholly adelgid threatens the hemlock. Human suppression of fire in the United States has so disrupted natural ecosystems based upon repetitive small fires that many areas are threatened with

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[1] Maurice K. Goddard Professor of Forestry and Environmental Resources Conservation. I would like to thank Emily Lisy for her assistance in research for this article.

[2] 42 U.S.C. §§ 4321-4370f.

[3] These include, *inter alia*, the National Forest Management Act, 16 U.S.C. §§ 1600-1614, the Federal Land Policy and Management Act, 43 U.S.C. §§ 1701-1785, and the Wilderness Act, 16 U.S.C. §§ 1131-1136.

[4] *See* 40 C.F.R. § 1504(d).

[5] 16 U.S.C. §§ 1531-1544.

wildfires that would destroy the system rather than restore it. In these cases, human action is required to restore or maintain the balance.

The presumption against action, coupled with the requirement for comprehensive study and planning before taking action, can be particularly problematic for restoration actions. The complexity of natural systems and limitations on knowledge often makes it difficult or impossible, as a practical matter, to develop a comprehensive plan of action *ab initio* in cases of restoration. In natural systems, restoration often requires adaptive management using the process of circumscribed trial and error followed by modified trial and error.

Without the creation and consistent application of exceptions to the no action presumption of current federal laws, land managers can feel that their hands are tied and they are unable to undertake the required restoration actions quickly. Even where exceptions are applied, land managers may act at the risk that a federal court will second guess their action. Because additional study entails both cost and delay, requirements for study before restoration can deter any ameliorative action altogether or delay it until a species has become threatened or endangered and weathered the storm of listing under the Endangered Species Act. Where other political pressures demand some action, Congress has therefore occasionally intervened to provide relaxed or expedited standards to allow restoration programs. For example, in 1982, Congress created the experimental population concept in ESA to allow more flexibility to encourage or assist programs for reintroduction of threatened or endangered species.[6] More recently, in 2003, Congress enacted the Healthy Forests Restoration Act[7] to relax certain procedural requirements applicable to efforts to reduce excess fuel in forests using logging to prevent fires that threaten fire ecosystems as well as human homes (creating the political demand for some action).

These measures have been controversial. Moreover, at best, they represent Band-Aids dealing with limited aspects of a more pervasive problem that will emerge more frequently as human populations and influences increase outside of reserved forest and parkland areas. A more systematic approach is required to deal with the issue of actions to restore damaged species and ecosystems on federal lands. An opportunity now exists to develop a more systematic approach in the context of implementing the recommendations contained in the NEPA Task Force Report to CEQ: Modernizing NEPA Implementation (CEQ 2003). That report called for measures broadening use of exceptions to the presumptions against action and establishment of an adaptive management work group to explore increased use of adaptive management techniques. The experience and challenges posed by American chestnut restoration could inform this process. This paper will, therefore, examine the problems with the existing federal model and possible solutions in the context of the efforts of the American Chestnut Foundation to restore the American chestnut as a dominant forest tree in the east.

The American Chestnut Restoration Efforts

The American chestnut (*Castanea dentata* [Marsh.] Borkh.) was, for the last 2000 years, a major component of the forests of eastern North America. Its range extended from central Alabama north to Vermont, New Hampshire and Maine and west from southern Ontario through Ohio, southern Indiana, Kentucky, and Tennessee (Russell 1987). It was a co-dominant in the Oak-Chestnut Forest (now known as the Oak Hickory Forest) that formerly covered much of the northern and eastern Appalachian region (Kircher & Morrison 1988 at p. 58). In the heart of its range, the chestnut could comprise 25 to 50% of

[6] 16 U.S.C. § 1539(j); Endangered Species Act Amendments of 1982, Publ. L. No. 97-304, 96 Stat. 1411, 1422, § 6; *see* H.R. Report No. 97-567, at 17, 33-35, *reprinted at* 1982 U.S.C.C.A.N. 2807, 2817, 283333-2835.

[7] Pub. L. 108-148, 117 Stat. 1888 (Dec. 3, 2003), *codified at* 16 U.S.C. §§ 6501-6591.

the forest cover (Oak 2002; Rhoades 2001; Russell 1987). It represented up to 70 percent of the wood volume on some slope forests. (Paillet 2002).

As a dominant species, it played an important ecological and economic role. Its nuts were prolific and consistently produced. As such, they provided mast supporting many species of wildlife (Wright & Kirkland 1999-2000; Lord 1998-1999; Morgan & Schweitzer 1999-2000). It was a valuable source of lumber for furniture, construction fencing and poles, and was used in tanning (Buttrick 1915; Russell 1987). Its nuts were also widely used and marketed for a human food, being widely used for chestnut stuffing (Buttrick 1915). According to the author's grandfather, its nuts were sweeter and much tastier than the chestnuts available today.

In 1904 the fungus, *Cryphonectria parasitica* (Murrill) Barr, was introduced from Asia to the Bronx New York, causing the chestnut blight (Oak 2002). The blight rapidly spread throughout the range of the American chestnut, killing 50 to 99 percent of American chestnut trees by 1940 (Oak 2002). Today, the American chestnut has been virtually extirpated throughout its range as a canopy tree.

The American chestnut persists as an understory or shrub species as a result of root sprouting. Virtually all trees produced from sprouting are eventually killed by the blight, although a few persist to produce some seeds. As a result of its sprouting behavior, the American chestnut "is in a somewhat unique situation among candidates for species restoration" in that "probably millions of sprouts" remain, despite the extirpation of the tree. As a result, the chestnut "is ranked G-4" "widespread, abundant and apparently secure globally" but presenting "some cause for long-term concern" (Irwin 2003).

The disappearance of the chestnut as a canopy tree, nevertheless, has had "profound" ecological implications (Oak 2002). Its disappearance has been implicated in the decline of oaks, particularly in the southern Appalachian region (Oak 2002). Because its nut production was more prolific and reliable and its nuts more nutritious than its replacement, the acorn, its disappearance, like the disappearance of other keystone species, has likely had wide implications for many wildlife species (Lord 1998-1999). The decline of the Allegheny woodrat (*Neotoma magister*), a species that has been federally listed as endangered, has been attributed to the disappearance of the American chestnut (Wright & Kirkland 1999-2000). Numbers of eastern wild turkeys have also likely been depressed (Morgan & Schweitzer 1999-2000).

The importance of the chestnut to human and natural systems has created incentives for several programs that hope to restore the American chestnut by creating blight resistant strains and reintroducing these strains throughout the chestnut's former range. Although these programs are collaborative and involve the joint efforts of individuals and private institutions, universities and state and local government agencies, the efforts have been privately led.

Four restoration methods are being pursued. The first effort, which is described here at length and appears poised to begin implementation of the actual restoration effort, is that of the American Chestnut Foundation; that effort involves backcrossing American chestnuts with oriental chestnuts to incorporate blight resistance. A second effort, which also appears promising, is using recombinant techniques to attempt to incorporate blight resistance directly into the DNA of the American chestnut. The American Chestnut Cooperators' Foundation is involved in a program of breeding the more blight resistant American chestnuts that can be found in the wild. Other efforts seek to utilize viruses that cause the blight fungus to become hypovirulent.

The American Chestnut Foundation was founded in 1984 to pursue the backcross restoration method. The Foundation's efforts have now approached the point where reintroduction and restoration of American chestnut appears feasible in the near future. The Foundation has established a program which

involved crossing the American chestnut with the Chinese chestnut and then backcrossing the progeny (F_1) with American chestnuts found flowering in the wild three times. After each backcross, the Foundation selected only the trees with blight characteristics and the phenotypic characteristics of the American Chestnut. This has produces trees that are genetically 15/16 American and blight resistant. The F_1- B_3 generation is then intercrossed two times, again selecting for blight resistance and the American chestnut phenotype, to produce offspring that are homozygous for resistance and otherwise include predominantly American chestnut genes. The objective of this program is to produce "backcross trees [that] will fall within the range of American chestnut taxonomic characteristics as understood from monographs and voucher specimens, [although] known to carry alleles from Chinese chestnut. [Its hybrid origin will not be recognizable except for its] blight resistance and on a DNA level." This breeding program has now produced the B_3-F_2 trees for seed orchards that will produce the B_3-F_3 nuts that will be planted in the forests as part of the final restoration effort, described below (Hebard 2002, 2003; Irwin 2003; Burnham 1991).

The next step in this effort will entail reintroducing American chestnut into the forest by planting B_3-F_3 seedlings from the seed orchards. How this will proceed has not been settled, but it will involve a series of steps employing adaptive management techniques. It will likely first entail installing experimental small plantations on existing openings on federal, state and other public or institutional lands. These plantations and any impacts will be monitored. Moreover, since the ecology and needs of the American chestnut are not fully known, a certain amount of experimentation will be needed to determine optimum planting techniques, favorable soil characteristics and the need for management techniques, including possibly controlled burning (Klinger 2000; Perry 2003) The American chestnut is released in sunlight, so the creation of clearings in forests will likely be required. After the plantations prove successful, the reintroduction will occur on a larger scale.

Resistant chestnuts created by other methods for will also likely be poised for use in reintroduction in the near future. Genetically engineered American chestnuts containing genes that may create blight resistance have also been created by the second method and may be ready for introduction by the end of the decade. Inoculation of American chestnuts with the hypovirulent virus may also proceed in federal lands.

The introduction of the backcrossed American chestnuts and other resistant strains can proceed without procedural impediments on state, institutional and private lands. However, it may run afoul of the federal presumption against action when attempted on federal lands. As discussed more fully below, existing law provides mechanisms that ought to allow the flexibility to allow these efforts to proceed without significant delay or cost. However, third party litigants who are concerned about possible adverse effects, the courts, and land managers who are concerned about the threat of litigation or otherwise unwilling to depart from standard operating procedures may impose costly environmental impact study procedures that could slow reintroduction efforts on federal lands or make some infeasible. A consistent federal policy designed to reverse the presumption against taking action in cases of reintroduction and restorations could help avoid these costs and delays and better serve the underlying intent of NEPA and other federal laws intended to incorporate environmental concerns into federal decision making.

What is Needed in a Restoration Effort

In assessing how federal laws might be applied to restoration efforts and what form a federal restoration policy might take, it is helpful first to consider the characteristics of an effective restoration project. These characteristics have described by a variety of authorities in a variety of contexts (Adams *et al.* 1998; Frelich & Puettman 1999; Harker *et al.* 1999; Henry & Lucash 2000-2001; Gjerstad, D. 2000-

2001) and can be applicable to efforts to restore the American chestnut (Craddock, J. H. 2000-2001; Irwin 2003).

All restoration programs require affirmative actions to reintroduce a species, to restore habitat or site conditions, and to manage the species and site after introduction both to maintain the species or system that has been restored and to make any changes found to be necessary. Physical site modification and planting or release of native or formerly native species will be required and physical management to maintain or restore soil conditions or control plants or animals following introduction will usually be required.

In most cases, reintroductions must be initiated with limited knowledge of the ecology, threats and requirements of the species or system to be restored. This requires that any restoration be preceded by study of the historic or paleohistoric records. The American Chestnut Foundation has been gathering this information since its inception. However, much of the information is simply unavailable and cannot be obtained without actual experience in the field.

Adaptive management techniques will be required throughout the reintroduction effort. Reintroduction, itself, will require at least two steps. The initial step of limited reintroduction or restoration will often involve planting test plots or releasing a limited number of individuals in somewhat controlled conditions and monitoring these areas. The American Chestnut Foundation has developed seed orchards for its backcrosses using several strains of American chestnut, with the American chestnut genetic material being gathered from a variety of trees in several different regions. Trees from seed orchards containing the "local" genetic material will be planted in these test plots. In this step, information on techniques for restoration, the needs of the species or system being restored, management techniques, possible threats to the restoration and impacts, if any, of the restoration can be identified. The techniques and management can then be adapted to structure the actual reintroduction.

The actual reintroduction will involve planting the American chestnuts at sites throughout each relevant region to encourage the widespread introduction. This step will involve site selection, site preparation and planting guidelines based on the experience in the test plots. The reintroduced species will require monitoring and will likely require management. For example, fire likely played an important role in chestnut ecology, such that controlled burns may be required for site preparation and management (Perry 2003). Use of herbicides to control competing vegetation may also benefit American chestnut restoration. Monitoring will be required to determine issues such both the success of the reintroduction, and the genetic and phenotypic characteristics of new trees, the impacts of the reintroduction and possible modifications of management techniques. Corrective actions will likely often be required.

Legal and Practical Barriers to Reintroduction on Federal Lands

Although the basic structure of federal environmental laws and regulations governing the use of our public lands begins with the presumption that new actions should be deferred and studied pending implementation, these laws and regulations provide sufficient flexibility to structure a program that will allow restoration programs such as that proposed for the American chestnut to be readily implemented without excessive cost or delay. In many cases, however, the opportunity to use this flexibility is squandered due to the unwillingness of federal managers or regulators to take advantage of these opportunities, whether due to fear of making a mistake, an unwillingness to depart from standard operating procedures, or concern regarding possible litigation. Flexibility can also be hampered by litigation brought by groups equally unwilling to depart from standard operating procedure, often due to suspicion regarding the motivation of the federal managers or, more frequently, their political superiors.

Courts, too, contribute to this confusion, upholding actions to expedite restoration activities in some cases and halting these activities to require additional costly process in virtually identical situations.

These conflicting results could be avoided with the formal adoption of a consistent federal policy applicable across all agencies towards the treatment of species or ecosystem restoration plans under federal law. Sufficient flexibility likely exists within the existing statutory framework to allow such a policy to be implemented by regulation, Executive Order or as formal guidance. The new federal policy should explicitly provide flexibility and encourage immediate implementation of restoration actions employing adaptive management under existing law, regulation and guidance. Adoption of a consistent, interagency, written policy would have several advantages. It would limit the discretion of the federal officials unwilling to take a risk or depart from standard operating procedure. It would provide written direction to courts that would help avoid the types of conflicting results that typify the current legal landscape and support the concerns of the federal officials whose inaction so often stymies proactive restoration. Finally, if it includes adequate safeguards against abuse, it might serve to alleviate the concerns among the groups who bring the litigation. Care must be taken in crafting such a policy to assure that it is, not, in fact, subject to abuses, but too much care in that regard could eviscerate the intent of the policy. The outline and justification for such a policy, using the American chestnut restoration as a model, are provided here.

Several environmental laws are applicable or potentially applicable to implementation of the American chestnut restoration on federal lands or similar efforts. As the fundamental environmental law governing all government planning and any federal action, NEPA[8] can apply and will be the focus of this article. The National Forest Management Act ("NFMA"),[9] although potentially applicable on National Forest lands used in the effort, will likely apply only in the context of NEPA. The Endangered Species Act ("ESA")[10] although inapplicable to the American chestnut restoration, is the federal law most applicable to restoration efforts and will be discussed because of the experience that has been gained under ESA regarding some of the difficulties in applying reforms encouraging voluntary action. A variety of federal laws are potentially applicable to the introduction of genetically engineered blight resistant American chestnuts, depending upon the genes introduced. The Federal Insecticide, Fungicide and Rodenticide Act ("FIFRA")[11] is potentially applicable to the efforts to use of the virus to induce hypovirulence in the chestnut blight fungus. The laws governing genetically modified organisms ("GMOs") and pesticide regulation are beyond the scope of this article.

NEPA - Establishing the Structure of Environmental Decision-Making Governing Restoration.

The National Environmental Policy Act has often been described as the foundation or the cornerstone of modern American environmental law. Enacted in 1970, it was the first major federal environmental law enacted in the "environmental decade" and has profoundly affected the development of environmental policy. It established:

> the continuing policy of the Federal Government . . . to use all practicable means and measures . . . in a manner calculated . . . to create and maintain conditions under which man and nature can exist in productive harmony. . .[12]

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[8] 42 U.S.C. §§ 4321-4370f.
[9] 16 U.S.C. §§ 1600-1614.
[10] 16 U.S.C. §§ 1531-1544.
[11] 7 U.S.C. §§ 136-136y.
[12] 42 U.S.C. § 4331(a).

NEPA's basic structure is simple. It seeks to require all government agencies to incorporate environmental consideration into all aspects of their planning, using an interdisciplinary approach. It seeks to do this through two mechanisms. First, it requires that each federal agency to use an interdisciplinary approach to incorporate environmental considerations into its planning and "include in every recommendation or report on proposals for legislation and other major Federal actions significantly affecting the quality of the human environment, a detailed statement by the responsible official on," *inter alia*, the environmental impacts, unavoidable adverse environmental effects, and alternatives to the proposed actions.[13] The United States Supreme Court has noted:

> NEPA has twin aims. First, it "places upon an agency the obligation to consider every significant aspect of the environmental impact of a proposed action." [citation omitted] Second, it ensures that the agency will inform the public that it has indeed considered environmental concerns in its decisionmaking process. [citation omitted]. Congress in enacting NEPA, however, did not require agencies to elevate environmental concerns over other appropriate considerations. [citation omitted].[14]

NEPA created the President's Council on Environmental Quality ("CEQ") to serve as an independent group overseeing all programs to assure consideration of environmental impacts of all federal policies. CEQ was given the authority oversee the environmental impact assessment requirements by promulgating rules governing agency implementation, overseeing agency implementation and resolving disputes. CEQ's regulations, thus, govern the structure under which restoration projects must be reviewed and assessed.[15]

CEQ regulations provide that agencies should "[i]nterpret and administer the policies, regulations, and public laws of the United States in accordance with the policies set forth in the Act and in these regulations."[16] Each agency must also adopt its own "procedures" incorporating the CEQ's requirements and supplementing those regulations as necessary.[17] The regulations call for "[i]ntegrat[ing] the requirements of NEPA with other planning and environmental review procedures required by law or by agency practice so that all such procedures run concurrently rather than consecutively."[18]

The central element of the NEPA process is the preparation of an environmental impact statement, assessing impacts across a range of media and concerns, developing alternatives, identifying mitigating measures and identifying unavoidable impacts.[19] Although the scope of the "statement" required by law is unspecified, in application, preparation of an EIS has become an expensive and time-consuming venture, involving analysis of multiple potential impacts and development of multiple alternatives. Moreover, these costly ventures often result in delay, since CEQ regulations mandate that an agency defer actions that will adversely affect the environment *or limit alternative choices* while the process is unfolding.[20]

Moreover, the EIS process, as applied, involves a top-down, comprehensive planning process that assumes that knowledge of impacts and effects of alternatives either exists or can be gathered before

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[13] 42 U.S.C. § 4332.
[14] *Baltimore Gas & Electric Co. v. Natural Resources Defense Council, Inc.*, 462 U.S. 87, 97, 103 S.Ct. 2246, 2252 (1983).
[15] These regulations appear at 40 C.F.R. Parts 1500-1517.
[16] 40 C.F.R. § 1500.2(a).
[17] 40 C.F.R. §§ 1505.1, 1507.3.
[18] 40 C.F.R. § 1500.2(c).
[19] 40 C.F.R. §§ 1501.7 (scoping); 1502.1-1502.25 (requirements for EIS preparation); 1503.1-1503.4 (public comment and response); 1505.2 (preparation of record of decision).
[20] 40 C.F.R. § 1506.1.

implementation. This assumption is invalid in many restoration projects, where use of adaptive management techniques may better serve the underlying statutory intent of environmental protection.

NEPA is intended to be a tool to assure that environmental concerns are incorporated into decision-making, not a mechanism to slow down and make actions more costly. CEQ has, therefore, by regulation, established several mechanisms to expedite the NEPA process, and federal agencies have incorporated these elements into their regulations, guidance and procedures. Thus, before undertaking an EIS, in many cases, agencies will undertake a less costly and less time consuming "mini-EIS" known an environmental assessment. This is described by one court considering a private proposal involving a mechanism reducing conflicts between private development and efforts to restore endangered species, as follows:

> NEPA is not designed to prevent all possible harm to the environment; it foresees that decisionmakers may choose to inflict such harm, for perfectly good reasons. Rather, NEPA is designed to influence the decisionmaking process; its aim is to make government officials notice environmental considerations and take them into account. By regulation, an agency considering whether an action would require preparation of an EIS must prepare a brief, preliminary evaluation, called an environmental assessment ("EA").[21]

The CEQ regulations also require all federal agencies to designate categories of actions that "do not normally require either an environmental impact statement or an environmental assessment."[22] A number of these "categorical exemptions" appear to apply to the American chestnut restoration and could enable these efforts, despite the overall presumption in favor of inaction.

Agency Procedures Relevant to Restoration: NEPA Compliance and Categorical Exclusion as Applied to the American Chestnut Restoration Efforts on Federal Lands

Although many federal agencies own and manage lands that may be involved in restoration efforts, most federal lands that might be involved in restoration efforts for the American chestnut will be managed by either the National Park Service ("NPS") or the United States Forest Service.[23] Accordingly, the impact of NEPA and the federal presumption against action must be examined in the context of the regulations and guidelines of those two agencies. These regulations and guidelines provide sufficient flexibility to authorize the initiation of restoration employing adaptive management avoiding the presumption against initiating actions without delay. However, as will be discussed further below, they do not provide sufficiently clear or consistent guidance to avoid the risk that these efforts could be derailed by public opposition, concerns of individual agency personnel or litigation.

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[21] *Center for Biological Diversity v. United States Fish & Wildlife Service*, 202 F. Supp. 2d 594, 647 (W.D. Tex. 2002); *see* 40 C.F.R. §§ 1501.4(b).

[22] 40 C.F.R. §§ 1507.3(b)(2)(ii); 1508.4.

[23] Most other significant federal lands fall under the jurisdiction the Bureau of Land Management (BLM"). However, lands under BLM jurisdiction are found primarily in the West, outside of the range of the American chestnut. The Department of Defense also manages extensive lands held as military bases, training areas and target and bombing ranges. DOD has developed a program for ecosystem management for its lands with the assistance of The Nature Conservancy (Leslie *et al.* 1996). However, DOD has not yet been actively engaged in the American chestnut restoration project. Moreover, DOD has a different mission than land management agencies and faces less significant budget constraints than do other land managers, such that it can readily retain outside contractors, such that delay and cost is less likely to deter restorative action.

NPS Procedures and Categorical Exclusions: The Management Policies of the National Park Service incorporate a variety of policies that encourage ecosystem and species restoration and would facilitate the reintroduction of the American chestnut on Park Service Lands (US Dep't of Interior 2000). These policies appear to reverse the general presumption against action for restoration and could serve as the kernel of a more general model.

The Park Service policies encouraging restoration arise from the mandate contained in the National Park Service Organic Act that the Service

> promote and regulate the use of . . . national parks, monuments, and reservations. . . by such means and measures as conform to the fundamental purpose . . .to conserve the scenery and the natural and historic objects and the wild life therein . . .and by such means as will leave them unimpaired for the enjoyment of future generations.[24]

The Park Service has broadly interpreted the non-impairment mandate to include any impact that would impair resources including past and external impacts and to require monitoring and affirmative action to address those impacts.[25] The Service's prohibition against intervention in natural or physical processes excludes efforts "[t]o restore natural ecosystem functioning that has been disrupted by past or ongoing human activities;" and the Service has adopted a restoration policy mandating action unless otherwise directed:

> The Service will re-establish natural functions and processes in human-disturbed components of natural systems in parks unless otherwise directed by Congress ...Impacts to natural systems resulting from human disturbances include the introduction of exotic species; the contamination of air, water, and soil; changes to hydrologic patterns and sediment transport; the acceleration of erosion and sedimentation; and the disruption of natural processes. The Service will seek to return human-disturbed areas to the natural conditions and processes characteristic of the ecological zone in which the damaged resources are situated. The Service will use the best available technology, within available resources, to restore the biological and physical components of these systems, accelerating both their recovery and the recovery of landscape and biological-community structure and function. Efforts may include, for example:
>
> - Removal of exotic species;
> - Removal of contaminants and non-historic structures or facilities;
> - Restoration of abandoned mineral lands, abandoned or unauthorized roads, areas over-grazed by domestic animals, or disrupted natural waterways and/or shoreline processes;
> - Restoration of native plants and animals.[26]

The Service's Policies further encourage establishment of public-public and public-private partnerships to accomplish these goals.[27]

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[24] 16 U.S.C. § 1.

[25] U.S. Department of the Interior, National Park Service, Management Policies 2001 ("*NPS 2001 Policies*"). NPSD1416, §§ 1.4.5, 1.4.7, 1.5(2000). The Park Service is in the process of updating its Management Policies, 70 Fed. Reg. 60852 (October 19, 2005), *see* U.S. Department of the Interior, National Park Service, Draft 2006 NPS Management Policies ("*Draft NPS 2006 Policies*"), *found at* http://parkplanning.nps.gov/document.cfm?projectId=13746&documentID=12825 (last visited October 20, 2005), §§ 1.4.4 - 1.4.7.

[26] *NPS 2001 Policies, supra,* §§ 4.1.5, 4.1; *see also, id.* § 4.4.1. These policies remain substantially unchanged under the proposed revisions to the policies. *See Draft NPS 2006 Policies, supra,* §§ 4.1.5, 4.1; *see also, id.* § 4.4.1.

[27] *NPS 2001 Policies, supra,* §§ 4.1.4, 4.2; *Draft NPS 2006 Policies, supra,* §§ 4.1.4, 4.2.

The NPS management principles broadly encourage restoration of natural populations, genetic diversity for those population and natural system and include specific standards governing restoration of extirpated species.[28] Fire management is encouraged and integrated pest management is allowed to protect rare, threatened and endangered or unique populations. The guidelines' requirement to restore native species and to remove or exclude exotic species creates some ambiguity for restoration of the American chestnut using the back-cross or genetic engineering methods, in that the Chinese chestnut genes might be considered exotic. However, the facts that, even in the back-crosses, the exotic genetic materials will constitute less than 10% of the genes and the plants will phenotypically be American chestnut, suggest that the new species should better be considered native. Moreover, the guidelines specifically provide that an exotic species may be introduced where all measures have been taken to minimize harm and the species is "[a] closely related race, subspecies, or hybrid of an extirpated native species; or [a]n improved variety of a native species in situations in which the natural variety cannot survive current, human-altered environmental conditions; or [u]sed to control another, already-established exotic species."[29] These exceptions, coupled with the mandate to restore systems and species would seem to encompass all of the methods for chestnut restoration.

The Park Service has incorporated its presumption in favor of restoration activity into its NEPA policies. However, these policies are not as well developed as its management guidelines. The Park Service categorically excludes a variety of actions related to restoration projects from requirements for an EIS or EA. These include: "[d]esignation of environmental study areas and research natural areas," "[s]tabilization by planting native plant species in disturbed areas," "[r]estoration of noncontroversial native species into suitable habitats within their historic range and elimination of exotic species," "removal of park resident individuals of non-threatened/endangered species which pose a danger to visitors, threaten park resources or become a nuisance in areas surrounding a park, when such removal is included in an approved resource management plan", and grant programs related to these activities.[30]

Forest Service Procedures and Categorical Exclusions: The Forest Service lacks a comprehensive restoration policy. The Service has a native plants policy, favoring use of native plants. The Service has also established the following categorical exclusion from NEPA documentation for planting native species:

> Regeneration of an area to native tree species, including site preparation which does not involve the use of herbicides or result in vegetation type conversion. Examples include but are not limited to: a) Planting seedlings of superior trees in a progeny test site to evaluate genetic worth and b) Planting trees or mechanical seed dispersal of native tree species following a fire, flood, or landslide.[31]

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[28] *NPS 2001 Policies, supra*, §§ 4.4.1, 4.4.1.2, 4.4.2.2 (*see discussion, infra*); *Draft NPS 2006 Policies, supra*, § 4.4.1, 4.4.1.2, 4.4.2.2.

[29] *NPS 2001 Policies, supra*, §§ 4.4.4. The new guidelines allow introduction of "exotic" species where "when all feasible and prudent measures to minimize the risk of harm have been taken, and

- it is a closely related race, subspecies, or hybrid of an extirpated native species, or
- it is an improved variety of a native species in situations in which the natural variety cannot survive current, human-altered environmental conditions. . . *Draft NPS 2006 Policies, supra*, § 4.4.4.

[30] United States Department of the Interior, Department of Interior Manual, National Environmental Policy Act Implementing Procedures for the National Park Service, 516 DM 6, App.7, § 7.4E(3),(4),(6)(7), F(1), *found at* http://elips.doi.gov/elips/release/3511.htm.

[31] Forest Service Handbook ("FSH") 1909.15 - Environmental Policy and Procedures Handbook ¶ 31.2(5) , *found at* http://www.fs.fed.us/emc/nepa/includes/epp.htm#c31. By regulation, the Forest Service designates its NEPA procedures in the Forest Service Handbook. 36 C.F.R. § 200.4.

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[30] United States Department of the Interior, Department of Interior Manual, National Environmental Policy Act Implementing Procedures for the National Park Service, 516 DM 6, App.7, § 7.4E(3),(4),(6)(7), F(1), *found at* http://elips.doi.gov/elips/release/3511.htm.

[31] Forest Service Handbook ("FSH") 1909.15 - Environmental Policy and Procedures Handbook ¶ 31.2(5) , *found at* http://www.fs.fed.us/emc/nepa/includes/epp.htm#c31. By regulation, the Forest Service designates its NEPA procedures in the Forest Service Handbook. 36 C.F.R. § 200.4.

any other notice used to inform interested and affected persons of the decision to proceed with or to implement an action that has been categorically excluded.[35]

Alternative Federal Restoration Models

There are examples of alternative federal models that expressly apply to restoration actions and mandate action that has been categorically excluded from the predominant presumption against action. Restoration actions are authorized and even required without the NEPA procedures in the case of endangered and threatened species under the federal Endangered Species Act ("ESA")36 and for contaminated sites and spills under the Comprehensive Environmental Liability and Compensation Act ("CERCLA").37 Although these programs provide some examples of mechanisms whereby the delays and costs incident to the predominant model might be avoided while preventing abuses and still incorporating environmental planning and public participation into federal programs, they also provide examples of some of the pitfalls in trying to balance these considerations. In some cases, in trying to strike this balance, these programs, particularly the CERCLA program, have generated even greater costs and delays for some restoration programs. Both programs provide examples of the problems incident to the top down, comprehensive planning approach that predominates under the existing federal model. Finally, both provide examples of how even a well structured program can be undercut by the tendency of agency personnel or the courts to apply the standard model, notwithstanding contrary directions.

The Restoration Model under the Endangered Species Act: The federal Endangered Species Act ("ESA")[38] and the NEPA procedures and reforms developed by the United States Fish & Wildlife Service ("FWS")[39] provides an alternative to the predominant NEPA model, but also presents examples of some of the pitfalls of an alternative model. Under this ESA model, restoration projects for threatened or endangered species or their habitat is mandated and delays can be avoided. However, in the absence of clear guidance or policy, the policies to encourage rapid and flexible action have been hampered by unwillingness of individual agency personnel to depart from the traditional model, public controversy and inconsistent court rulings. Moreover, the "top down" and comprehensive planning approach envisioned under ESA makes its application to the American chestnut restoration and many other restoration programs problematic, at best.

ESA uses the "fine filter" approach to biodiversity conservation, seeking to protect biodiversity by protecting nationally endangered and threatened species and their habitat, with the stated purpose of conserving "the ecosystems upon which endangered species and threatened species depend."[40] ESA accomplishes this goal both by limiting actions and requiring affirmative restoration. Thus, ESA protects the individuals within threatened and endangered species directly, through its prohibition against "taking"[41] and requires the development and implementations of recovery plans for the species. Prohibited "takings" include activities which have an "incidental" adverse effect on a threatened or endangered species. However, the Act authorizes issuance incidental take permits allowing a property owner to conduct otherwise lawful activities in the presence of listed species, but requires each non-federal entity to develop an Habitat Conservation Plan calling for affirmative action to conserve the species or habitat. ESA also seeks to protect the land constituting critical habitat for endangered species.

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[35] *Id.*, ¶ 31.2.
[36] 16 U.S.C. §§ 1531-1544.
[37] 42 U.S.C. §§ 9601-9675.
[38] 16 U.S.C. §§ 1531-1544.
[39] The Fish and Wildlife Service and the National Marine Fisheries Service administer ESA.
[40] *Id.* § 1531(b).
[41] *Id.* § 1538.

To that end: (1) ESA requires that critical habitat be designated;[42] (2) the Act requires that each federal agency aid in the conservation of endangered species,[43] and assure that programs that it administers, including grant, permit, construction and management programs, will not jeopardize the continued existence of threatened or endangered species or "result in the destruction or adverse modification of" their critical habitat;[44] and (3) ESA requires both the United States Forest Service and the United States Department of the Interior ("DOI") to develop a broader affirmative program to conserve "fish, wildlife, and plants including those which are endangered species or threatened species" and authorizes those agencies to acquire land as a part of that program.[45]

ESA's affirmative obligations to develop and implement recovery plans and to conserve endangered and threatened species and their habitat represents a departure for certain restoration programs from the dominant federal presumption against taking affirmative action. Moreover, FWS has adopted a series of categorical exclusions which, on their face, would appear to allow a policy of permitting restoration activities to proceed without the delays and costs incident to development of either an EIS or EA. FWS' NEPA Guidance provides categorical exclusions for "[t]he reintroduction or supplementation (e.g., stocking) of native, formerly native, or established species into suitable habitat within their historic or established range, where no or negligible environmental disturbances are anticipated."[46] Similarly, FWS provides categorical exclusions for "restoration of wetland, riparian, instream, or native habitats, which result in no or only minor changes in the use of the Affected local area." Prescribed burning "for habitat improvement purposes" when carried out consistent with the law, fire management activities, is categorically excluded.[47] ESA and other FWS permit actions are excluded from NEPA requirements "when such permits cause no or negligible environmental disturbance." Incidental take permits that "individually or cumulatively, have minor or negligible effect on species covered in the habitat conservation plans as also excluded.[48] The issuance of recovery plans is excluded,[49] but any habitat conservation plan normally requires an EA under these procedures.[50]

Notwithstanding these departures from the predominant "wait and study" federal model, the structure of the ESA program suffers from a number of limitations, some of which have been addressed by recent reforms and some of which persist despite those reforms. ESA's "fine filter" approach leaves significant gaps in biodiversity conservation. It only addresses threatened and endangered species and does not address many important biodiversity features that require restoration. The frequency of the American chestnut's occurrence makes it less likely that it could be listed as threatened and endangered, despite the ecological importance of restoration of this keystone species as a canopy tree producing nuts. Moreover, ESA still envisions a top-down, comprehensive planning process that is inconsistent with the needs of many restoration projects. The listing process, requiring "best available science," is a prolonged and often contentious process that entails substantial delays. This has resulted in substantial backlogs that keep even those species eligible for listing off of the list for long periods of time. In theory, although not in practice, designation of critical habitat and recovery plans must be developed at the beginning of the process, when sufficient information is often unavailable, rather than through an adaptive management

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[42] *Id.* § 1533(a)(3).

[43] *Id.* § 1537(a)(1).

[44] *Id.* § 1537(a)(2); *Tennessee Valley Authority v. Hill*, 437 U.S. 153 (1978).

[45] 16 U.S.C. § 1534.

[46] Department of Interior Manual, National Environmental Policy Act Implementing Procedures for the Fish and Wildlife Services, 516 D.M. 6, Appendix 1, § 1.4 B(6), 62 Fed. Reg. 2375 (January 16, 1997), *also found at* http://elips.doi.gov/elips/release/3511.htm..

[47] *Id.*, 516 D.M. 6, Appendix 1, §§ 1.4 B(3), (4), (5).

[48] *Id.*, 516 D.M. 6, Appendix 1, §§ 1.4 C(1), (2).

[49] *Id.*, 516 D.M. 6, Appendix 1, § 1.4 D.

[50] *Id.*, 516 D.M. 6, Appendix 1, § 1.5.

process. Finally, ESA suffers from lack of flexibility, particularly with respect to the prohibition against takings, such that listing of a species would impair property uses and would create disincentives for private conservation efforts (Bean 2003; Taylor 2002). The flat prohibition against takings and requirements for incidental take permits, with the possible requirement for an EIS for such permits, also deters habitat restoration activities that would benefit the species in the long run but might incidentally "take" individuals.

Some of these problems were resolved by a number of reforms initiated under DOE Secretary Bruce Babbitt in the Clinton Administration (Bean 2003; Taylor 2002), some of which might facilitate the American chestnut restoration, could they be applied. The Candidate Conservation program seeks to encourage proactive programs to eliminate the necessity for regulatory controls by encouraging private conservation activity directed to unlisted species that would qualify for listing to reduce the threats to such declining species, and thus avoid listing. The requirements and procedures are incorporated into Candidate Conservation Agreements ("CCAs"); CCAs assure non-federal landowners that they can continue agreed-upon activities even if the species becomes listed in the future, and thereby avoid regulatory controls (Bean 2003; Ruhl 2004).[51] The program does not, however, apply to or encourage similar activities by federal landowners. Safe Harbor Agreements encourage voluntary actions by landowners to protect endangered species, in return for protection against future changes (Bean 2003; Taylor 2002; Ruhl 2004).[52] FWS has recently proposed amendments to its permitting rules that, if adopted, would provide greater flexibility for habitat enhancement activities both in these programs and enhancement programs on federal lands, allowing incidental takes incident to programs that enhance habitat.[53]

The Candidate Conservation with Assurances mechanism, coupled with the categorical exclusion of habitat conservation activities might be an ideal mechanism to encourage the restoration of the American chestnut, which might be in danger of becoming threatened. However, the policy does not apply to federal lands. Moreover, problems have emerged in the application of reforms. Whether because of unwillingness to depart from the traditional model for federal action or concern regarding judicial review, federal managers have often been unwilling to apply these models. Rather than expedite implementation, FWS has often delayed implementation with long review, excessive requirements in the agreements, and insistence on studies regarding impacts and effects. At times, reforms have been slowed by insistence on a showing that individuals not be taken, despite a clear expectation that the species would benefit from habitat improvement. This unwillingness to depart from business as usual has led some parties to abandon projects and others to indicate an unwillingness to use the reform mechanisms in the future (Bean 2003). Moreover, at times, the concerns of the managers have been confirmed by inconsistent results in the courts. *Compare Center for Biological Diversity v. United States Fish and Wildlife Service*, 202 F. Supp. 2d 594 (W. D. Tex. 2002) (upholding incidental take permit where permittee protected off-site conservation areas providing superior habitat for endangered species and rejecting claim that alternative reducing size of on-site disturbance should have been more fully developed and required) *with. Gerber v. Norton*, 294 F.3d 173 (D.C. Cir. 2002) (overturning incidental take permit where permittee protected off-site conservation area providing superior habitat, with court relying on failure to provide adequate comment and failure to adopt on-site plan that would minimize area of disturbance).

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[51] United States Department of the Interior, Announcement of Final Policy for Candidate Conservation Agreements with Assurances, 64 Fed. Reg.32,726 (June 17, 1999).

[52] United States Department of the Interior, Announcement of Final Safe Harbor Policy, 64 Fed. Reg.32,717 (June 17, 1999).

[53] United States Fish & Wildlife Service, Proposed Revisions to the Regulations Applicable to Permits Issued Under the Endangered Species Act, 68 Fed. Reg. 53327 (May 3, 2003).

The Restoration Model under the Comprehensive Environmental Liability and Compensation Act: The approach adopted by the United States Environmental Protection Agency ("EPA") in pursuing hazardous substances remediation under the Comprehensive Environmental Liability and Compensation Act ("CERCLA")[54] presents a useful model in two senses. The EPA model, as spelled out in the statute and the National Oil and Hazardous Substances Pollution Contingency Plan ("NCP")[55] provides an example of a case where the establishment of requirements and safeguards governing a restoration action can allow actions to proceed immediately without the delays built into existing statutes such as NEPA. The law and EPA regulations spell out the procedures required for restoration and NEPA compliance is not required, due to the fact that the procedures developed were found to offer equivalent protections. The experience under CERCLA and the NCP, however, also presents a model of mistakes to be avoided, in that, although those requirements allow immediate implementation, the procedures and safeguards required for longer term actions have been widely criticized as excessive, causing excessive cost and delay.

Under CERCLA, where there is an immediate need to proceed to prevent continuing harm, EPA may proceed immediately to implement a "removal action" without the extensive study and public participation required for a full "remedial action" that will achieve final cleanup.[56] If a planning horizon of greater than six months is required, somewhat more participation and study is required, more equivalent to an EA. However, response other than investigation and monitoring is limited to actions costing less than $2,000,000 and lasting one year unless EPA finds that continuing timely action is otherwise necessary to protect health or the environment.[57] In many cases, restoration will be achieved without further action. However, in other cases, a full Remedial Investigation and Feasibility Study ("RI/FS"), involving study of alternatives often greater than entailed in an EIS, is required. In its promulgation of the first version of the NCP, EPA determined that compliance with other federal laws, such as NEPA, was not required, but that these procedures created equivalent protections.[58] Other federal agencies pursuing cleanup pursuant to the NCP have consistently determined that NEPA compliance is not required.

The CERCLA determination offers a helpful precedent, not because that model should be replicated for species and ecosystem restoration, but because it presents a situation where an agency has determined that NEPA and other federal procedural requirements are inapplicable because the action at issue (1) involves restoration and (2) incorporates requirements that will prevent abuse. The CERCLA model for study and procedures, particularly for the RI/FS, however, is unduly costly and has been widely criticized for causing the undue costs and delay that undermine the statutory goal of achieving restoration quickly and effectively. The optimal restoration model would incorporate the CERCLA model of establishing safeguards to achieve the goals of permitting rapid and effective restoration and excluding these actions from procedural requirements such as NEPA, while not creating a whole new set of requirements for study and procedure that will create greater delay and greater cost.

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[54] 42 U.S.C. §§ 9601-9675.

[55] 40 C.F.R. pt. 300.

[56] 40 C.F.R. § 300.415.

[57] 42 U.S.C. § 9604(c).

[58] United States Environmental Protection Agency. 1985. Preamble. The National Oil and Hazardous Substances Contingency Plan, 50 Fed. Red. 47912, 1985 WL 126730 (Nov. 20, 1985). In the 1986 Amendments to that CERCLA, Congress further spelled out where compliance with other laws was required, specifying that permits not be required, 42 U.S.C. § 9621(e) but requiring that applicable and appropriate substantive (but not procedural) requirements apply to final cleanups, *id.* § 9621(d).

Problems with Existing Mechanisms to Facilitate American Chestnut Restoration

The existing categorical exclusions in Park Service and Forest Service policies should allow reintroduction and restoration to proceed using adaptive management techniques without the need for an EIS or EA. However, there are a number of potential problems that could arise to inhibit that action. These problems could also inhibit other types of ecological restoration programs.

The existing exclusions are far from uniform. Moreover, they are not uniformly applied in the field. This is likely due to the lack of any clear standards or uniform policy governing ecological restoration on federal lands. Without clear guidance, as under ESA, agency personnel will often fall back on the more traditional "wait and study" approach that will delay and may prevent restoration actions.

There is also often a lack of trust that is exacerbated by a lack of clear, transparent standards. The public often mistrusts the government. Moreover, stakeholder groups mistrust one another. Without clear guidance indicated when and how a restoration action will take place, these groups may demand more process and study. These demands may further induce agency personnel to delay action and study the problem further. The lack of trust may also generate litigation when the agency does proceed with a restoration action which raises a concern for some interest group.

The lack of clear guidance and a clear policy favoring restoration action over the 'no action' alternative will also affect the courts. In the absence of a clear articulated and clear policy, the courts will also fall back on the traditional model. In fact, the judicial reliance upon precedent may force such a result without clear guidance on an alternative model that has been adopted by the agencies. This lack of guidance will create inconsistent results, as have appeared in the application of the ESA reforms noted above.

All of these threads are apparent in appeals from actions of the Forest Service. The lack of trust noted above has generated an explosion of litigation, including litigation against restoration programs. Between 1997 and 2002, 3737 appeals from Forest Service action were filed before the United States Courts of Appeals, including 139 appeals challenging restoration programs and 97 challenging prescribed burn programs often necessary for ecological restoration and possibly necessary for American chestnut restoration (Malmsheimer *et al.* 2004). This explosion of litigation, in part, motivated Congress to pass the Healthy Forests Restoration Act[59] to allow restoration activities employing logging in order to address the adverse impacts of years of fire suppression.

It is entirely possible and even likely that the proposals of the American Chestnut Foundation to proceed with its American chestnut restoration on Park and Forest Service lands using adaptive management techniques will be able to proceed without an EA or EIS under existing categorical exclusions and without controversy or litigation. It is a popular native species unlikely to arouse opposition. Nevertheless, there are some characteristics of the restoration that could generate controversy and, in turn, inhibit both reliance on the categorical exclusion and efficient, early restoration action.

It is possible that some stakeholders could contend that the backcrossed American chestnut is a new species and possible, but less likely that those concerned about alien invasive species could object to the reintroduction. Such an objection bears some risk of bringing the reintroduction program outside of the Park Service's categorical exclusion, which applies to "noncontroversial" native species. However, the facts that oriental chestnuts and hybrids have been widely introduced in the past and that the current backcross is phenotypically and largely genetically American chestnut make it unlikely that such an objection could prevail.

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[59] Pub. L. 108-148, 117 Stat. 1888 (Dec. 3, 2003), *codified at* 16 U.S.C. §§ 6501-6591.

The foregoing possibilities raise greater concern for the restoration of the genetically engineered American chestnut, even though the genetic makeup of that species would be American chestnut, but for four genes. Genetically modified organisms have spawned significant controversy, particularly in Europe. Although the relatively insignificant modifications introduced by genetic engineering make it less likely that the blight-resistant American chestnut could be considered a "new" species, significant controversy could deter either agency's willingness to rely on categorical exclusions. Moreover, litigation is often a throw of the dice, particularly in the absence of clear written guidance.

Preparation of sites for planting blight resistant American chestnuts and management activities may also be unnecessarily restricted by limitations on the categorical exclusions. Because American chestnut requires sun, site preparation may require canopy opening *(i.e.* logging), which could arouse opposition or litigation. The amount of canopy opening may be limited. Use of herbicides and fire management would assist establishment of American chestnut and suppression of competing species. Deer management will be required. Many planting sites might be restricted. For example, chestnuts frequently appeared on slopes. Some of these methods might be deemed to bring the restoration efforts outside of the Forest Service categorical exclusions.

On balance, though, existing categorical exclusions should support a properly managed program, if constrained, restoration of the American chestnut. Nevertheless, there is a risk of some controversy. These risks and the often unnecessary limitations upon the existing categorical exclusions suggest that a more consistent and unambiguous restoration policy will be useful. These risks could generate even greater risks for other restoration efforts, which would also benefit from a firm interagency federal policy favoring action on restoration over inaction, embodied in regulation or clear policy cutting across agency jurisdictions and programs.

An Alternative Model For Environmental Restoration Projects

An alternative to the current model that would better advance the underlying intent of NEPA in addressing ecological restoration activities would treat the "restoration action" in the same manner as the "no action" alternative to treated in other cases, allowing that action to proceed while requiring a justification for failing to act. This alternative could be achieved by regulation or by policy, including a policy regarding categorical exclusions. It could also be achieved through appropriate legislation.

While this approach is conceptually simple and consistent with the intent of NEPA, it becomes somewhat more difficult in application. The "no action" alternative is readily defined as "doing nothing" or "carrying on as usual." However, there is an infinite variety of positive actions that one could take. Thus, one must define what type of action constitutes a restoration action. This requires, initially, a definition of the goal of the action. It also requires definition of the quality of the action - - the action must be reasonably calculated to achieve that goal (Henry & Lucash 2000-2001).

A clear and somewhat limited definition of a restoration action is also necessary to prevent abuse. Many actions to advance interests other than restoration of the environment can be pretextually labeled as a "restoration" action. For example, many of the concerns regarding removal of accumulated fuel in forests arise from the fear that other forest harvests will be dressed up as fuel reduction programs where the real intent is to maximize profit by maximizing the amount of wood harvested. On the other hand, going too far to address these concerns can result in excessive restrictions which undermine the intent of expediting restoration, as has occurred under the CERCLA program.

Finally, some limitation in the types of restoration action that should proceed rather than waiting and studying alternatives is warranted in cases where the restoration may have an adverse impact on other important values. In these cases, a collision with other values may favor a more deliberative, if costly process. For example, where predator reintroduction would threaten human safety or threaten major impacts on economic interests, more study can be necessary before proceeding.

Many of the differences in categorical exclusions found in existing agency procedures may be explained as each individual agency's response to the foregoing concerns. However, the existing approaches are *ad hoc* and often inconsistent. We need more consistent, focused approach. Some guidelines for such an approach are suggested below.

The presumption that a restoration action should be treated as a "no action" situation and proceed would be triggered upon a finding that the procedure has certain characteristics. The categorical exclusions established by the Fish and Wildlife Service for restoration projects and the Park Service Guidance on restoration projects[60] include criteria that might be applied. The first such criterion should relate to the objective of the project. A restoration action should have the goal of restoring a native species or habitat that has existed in the site during historic times that has been removed or adversely affected by human activities or the results of human activities. Restoration should not, however, be limited to cases where the harm has already occurred. It should also include actions to protect native species and habitats from harm that is reasonably expected. For example, removal of vegetation infected with sudden oak death (a disease that may also threaten chestnut restoration) should proceed immediately rather than waiting and studying the impact of removal. This should include efforts to allow systems to adapt to human induced changes that are inevitable, such as the changes that will likely be associated with human induced climate change. Because systems are in a state of change even absent human influence, the exception to the wait and study presumption should, initially be limited to restoration of ecosystems or species that were native in historic times. Whether restoration megafauna of the type that became extinct in the Pleistocene extinction, an issue that may face Park managers in the future (Flannery 2001, pp. 345-346), would be included should await development of further knowledge.

A second criterion should address the manner in which the action will be carried out. To proceed without an EIS or EA, the restoration action must be carried out in a manner consistent with accepted practices, given the present state of knowledge. This does not mean that success must be assured. Knowledge of restoration is necessarily limited. For that reason, to qualify for the "categorical exclusion, the restoration action should employ adaptive management. Procedures for monitoring, reassessment and adjustment should be in place. In other words, procedural mechanisms must be in place to assure use of the best science available rather than a determination of the answers before action.

Finally, limitations should be included to assure that restoration action will not create major adverse disruption of important or valued human or natural systems. The establishment of limitations presents the greatest challenge. Arguably, it is overprotective limitations that currently inhibit restoration actions and that have created many of the inefficiencies in the CERCLA program as well as the ESA. Any limitation should have a defined threshold and not use vague terms that provide insufficient guidance to courts and agency person. For example, federal restoration actions should be subject to the same types of controls that would apply to non-federal lands. Annual limitations on the size of areas in which all vegetation would be removed might constitute another type of limitation. The policy should be clear, however, that the presumption would favor restoration and that doubt should be resolved in favor of proceeding rather than vice versa. In some cases, time or cost thresholds may be appropriate, as is the case with CERCLA removal actions.

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[60] NPS Management Policies, *id.* § 4.4.2.2.

Thus, a qualified restoration action would be one where (1) the project was designed to restore a system or species that had been removed or adversely affected by human disturbance or other disturbance related to human activity, (2) the restoration must be reasonably calculated to address or reduce effects of that disturbance (Henry & Lucash 2000-2001), and (3) the project is supported by a reasonable management plan that, if implemented, will not result in a serious threat to human safety, other environmental resources or property that cannot be adequately compensated with money damages, (4) procedures had been established for monitoring and adaptive management, and (5) the project did not any specifically established limitation or threshold established to conserve other important values.

This approach is consistent with the recommendation of the NEPA Task Force Recommendations to the Council on Environmental Quality (CEQ 2003). That report recommended establishing an adaptive management work group to broaden use of that tool in NEPA implementation. It recommended broadening use of categorical exclusions, while incorporating monitoring and adaptive management to gather data regarding categorical exclusions. It also recommended better integrating NEPA into other programs including the Endangered Species Act consultation program. Adopting a consistent, interagency approach to restoration would be consistent with all these recommendations. It would also better effectuate the intent of NEPA that federal actions proceed in a manner that will protect and enhance the natural environment and encourage proactive, privately led programs such as the American chestnut restoration.

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FEASIBILITY OF LARGE-SCALE REINTRODUCTION OF CHESTNUT TO NATIONAL PARK SERVICE LANDS: SOME THOUGHTS

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Abstract: In the early 1900s, chestnut blight transformed the eastern deciduous forest by eliminating American chestnut as a dominant overstory species. Currently, reintroduction of American chestnut appears possible with blight-resistant chestnut hybrids soon becoming available from various breeding programs. Overcoming chestnut blight through integrating resistance from Chinese and Japanese chestnut into the American chestnut genome represents a crucial first step in the process of restoration. Successful reintroduction, however, requires consideration of site, seed source, seedling quality, and silvicultural requirements. In addition, there are other challenges in eastern forests that await resistant seedlings. Considerations for reintroduction procedures and potential problems are discussed.

Keywords: American chestnut / Chestnut blight / seedling establishment / reintroduction

INTRODUCTION

Reintroduction of blight-resistant chestnuts to eastern North American forests is one of the most anticipated events in natural sciences by the general public. Unique to chestnut is the formation of private nonprofit foundations whose mission is to develop planting stock resistant to the chestnut blight fungus [*Cryphonectria parasitica* (Murr.) Barr]. As predictions for the release of blight-resistant seeds and seedlings approach, attention should shift from blight resistance toward the ecological and silvicultural considerations that will also determine the success of reintroduction efforts. Detailed studies of forest ecosystems, e.g., Clements (1916), and North American forestry research (cf. Pinchot 1947) in general, were just occurring, as American chestnut [*Castanea dentata* (Marsh.) Borkh.] was being eradicated as an overstory species, so little is known about the establishment and growth of this species.

The feasibility of large-scale reintroduction of blight-resistant chestnuts requires a greater understanding of many factors. Aspects of chestnut establishment success include site selection, material selection, and anticipation of establishment challenges. Given the dearth of information on artificial regeneration of American chestnut, reintroduction strategy on National Park Service (NPS) lands should encompass existing information on establishment techniques on chestnut and closely relate genera, e.g., *Quercus* L., prior to or concurrent with the forthcoming release dates for hybrid material under the framework of existing natural resource management policy. This paper will consider some of the important factors that will affect reintroduction on NPS lands.

REINTRODUCTION CONSIDERATIONS

Site availability on National Park Service lands

Restoration of chestnut on federal and State land bases will be affected by land management policy for each respective agency. The forest is resilient in terms of response to disturbance, and the gaps left by

dead chestnuts have long been filled by other species. National Park Service lands are generally managed to mimic natural disturbance regimes, as opposed to USDA Forest Service lands that may be subjected to harvesting. The first priority for reintroducing chestnut is to identify the appropriate sites for establishment.

American chestnut is an intolerant species and requires a yet-undetermined amount of light to grow and successfully compete in eastern forests. Opening sites for planting blight-resistant chestnuts through harvesting is not a viable option for National Park Service lands. On larger NPS lands, suitable sites for planting chestnut can become available through the effects of natural disasters, fire, and pests on forested land. Tornados, straight-line winds, and hurricanes all can cause forest destruction by blowing down or shattering trees, thereby opening sites. Depending on the severity, fire can remove the overstory and understory forest on significant acreages. Natural and exotic pests can also create openings and gaps in which chestnut could be successfully established.

Seed Source Considerations

Conservation programs, aimed at increasing disease resistance can reduce genetic variation within the host species population. In the 1950s Forest Service breeding production programs for blister rust-resistant white pine in the western United States incorporated only 100 selected parent trees for reintroduction (Neuenschwander et al. 1999). The resulting seedlings, though disease-resistant, were limited in their "fitness" or ability to adapt to varying environmental conditions (Buchert 1994). The natural geographic range and range of forest sites of American chestnut is large and therefore, individual breeding programs for different regions need to be implemented.

The use of seed sources adapted to local conditions is an important factor in artificial regeneration success (cf. Zobel and Talbert 1984). When information about genetic variation and adaptability of the species are unknown, locally-adapted seed sources have proven to be importance for seedling survival and mast production (Wakeley 1963). Incorporating of locally-adapted genotypes into breeding programs is desirable, as the use of unproven genotypes can cause problems in:

- Short-term and long-term survival;
- Poor adaptability to local environment can mean poor productivity in terms of growth and frequency and quantity of mast production;
- Unproven genotypes could affect overall productivity of the forest through pollination of naturally occurring trees of the same species; and
- Planting of seedlings from unspecified seed sources could be challenged by private citizen, e.g., "environmentalist," groups

As National Park Service lands are managed as bioreserves, use of genotypes from the local gene pool in a breeding program is particularly desirable. Practicality, however, is another issue, as resources for separate breeding programs for each National Park Service land base is not feasible in the foreseeable future. Therefore, the critical question is, how far can seedlings of one breeding program be moved and still exhibit satisfactory survival and growth? In addition, there are significant environmental differences among chestnut sites within NPS lands with large acreages that can affect survival and growth.

In general, there are few guidelines for seed transfer in hardwood species (cf. Post et al. 2003) due to a relatively small amount of genetic testing done, in comparison to certain coniferous species. In the absence of these guidelines, identification of geographic areas with common environmental conditions, i.e., seed zones, can be useful to guide testing to determine seed transfer parameters. The wide range of physiographic and climatic conditions in Tennessee has lead to the creation of a hardwood seed-zone

system as a guideline for seed collection throughout the state (Post et al. 2003). This system was developed using a Geographic Information System (GIS) and was based on elevation, Bailey's Ecoregions, 30 years of monthly precipitation, and 30 years of monthly minimum temperature data. Similar models can be created for large NPS land bases in combination smaller, proximal NPS land bases to guide seed transfer testing.

It is possible that these models may be further refined by using a geographic information system (GIS) analysis that incorporates site information from historic and current chestnut sites. Butternut (*Juglans cinerea* L.) is an eastern hardwood species that is being decimated by a disease caused by an exotic fungus (*Sirococcus clavigignenti-juglandacearum* Nair, Kostichka, & Kuntz). Over 80 percent of the butternut population in southern States has been destroyed (USDA Forest Service). Surveys for surviving butternuts in the Great Smoky Mountains National Park, Mammoth Cave National Park, and St. Francis National Forest have been aided by predictive models generated by GIS analyses (van Manen et al. 2002; Thompson et al. 2004a.b).

Seed Production

Seed orchards will be necessary to generate the copious amount of chestnuts needed for reintroduction on NPS lands. Placement of seed orchards should be carefully coordinated with the NPS lands that they are intended to serve. Unpredictable expression (or lack of expression) of certain traits have been known to occur in progenies from seed orchards that were located remote to the intended areas of reforestation (Skroppa and Johnson 2000). Development of seed orchard management protocols will be another area that will demand attention. Management protocols for American chestnut orchards can be guided by the large volume of experience and research on several *Castanea* Mill. species (J.H. Craddock, Pers. Comm., May 1, 2004).

Development of pest management schedules specific for chestnut seed orchards will probably be needed, as North American pests are different from European and Asian pest species. Post et al. (2001) studied the effects of insecticide spraying in a *Quercus rubra* L. seed orchard and found that a number of seed pests attack acorns. A variety of other pest species were also found in this orchard (Schlarbaum et al. 1998), which indicates that a significant amount of research will be needed to keep chestnut orchards healthy.

In general, the NPS does not have the necessary expertise, suitable land, nor equipment for seed orchard development and management. Successful seed orchards require expertise in tree improvement, genetics, forest pathology, and forest entomology and well as a technical staff that is trained for working in highly managed conditions. Seed orchards require pesticide spraying and fertilization, which are not usually conducted on NPS lands. The NPS also does not generally have the type of equipment needed for seed orchard management. As seed orchard development becomes more eminent, the NPS should consider cooperating with USDA Forest Service Regional Genetic Resources Programs and State Divisions of Forestry (or equivalent), which have trained personnel in seed orchard management, equipment, and the land with appropriate variances for insecticide spraying, etc.

Hardwood Seedling Quality and Establishment

American chestnut has been planted since the mid 1800s (cf. Emerson 1846). By the 1880s, experiments in establishing American chestnut on the Great Plains had been conducted for a number of years (Egleston 1884). Detailed studies of growth after establishment are lacking from these early years, as forestry research, indeed forestry itself, was in an infantile state in North American during the latter stages of the 19th century (cf. Pinchot 1947). Because of the lack of information regarding chestnut seedling establishment, it is advisable to adopt guidelines from comparable hardwood species in order to develop

initial strategies to improve chestnut establishment. While survival rates and specific site and silvicultural requirements differ between oak species and chestnut, information on artificial regeneration of oak can provides useful information.

Establishment of seedlings from heavy-seeded hardwood species through natural or artificial processes in eastern hardwood forests is becoming increasing difficult in recent years. Invasions of sites by exotic plant and vine pests and a dramatic increase in white-tailed deer (*Odocoilens virginianns* Zimmerman) herds augment difficulties with suppression by faster growing light-seeded species and hardwood sprouts. Artificial regeneration protocols are needed to combat existing and growing challenges for successful establishment of blight-resistant seedlings on NPS lands.

Production of large, vigorous seedlings is a partial answer to these problems. Seedlings that are able to either maintain or grow sufficiently to keep the terminal bud/shoot above competition and above the level of deer browse will have an increased chance for survival and establishment. Nursery protocols developed by the USDA Forest Service's Institute of Forest Genetics and implemented by the Georgia Forestry Commission at the Flint River Nursery (Kormanik et al. 1993) have been shown to produce high quality 1-0 hardwood seedlings that are taller, thicker and have more robust root systems hardwood seedlings produced under standard nursery protocols. Studies with high-quality oak seedlings show a relative increase in establishment success (Kormanik et al. 2002). Chestnut establishment success will probably increase if seedling size is optimized.

Chestnut seedlings have responded favorably to protocols developed by Kormanik et al. (1993) at the Flint River Nursery and to an additional season of growth at in the northern Connecticut State Nursery. Some American chestnut seedlings (1-0) grown at the Flint State Nursery reached over 1.8 m in height, while some chestnut hybrid seedlings (2-0) were approaching 2 m tall at the end of a second growing season in Connecticut. There were genetic differences in nursery height, root collar diameter, and number of lateral roots among genetic families at both locations.

Plantings of the Georgia-gown seedlings in Kentucky incurred severe mortality and heavy competition (Brosi 2001). Mortality was primarily due to *Phytophthora cinnamomi* Rands., an exotic root rot disease brought into the country in the early 1800s (*teste* Clinton 1913) and chestnut blight, which had infected some seedlings in the nursery. Herbivory by deer also impacted the unprotected trees. Despite these problems and heavy competition from yellow-poplar (*Liriodendron tulipifera* L.), the surviving trees grew on average 40 cm a year. After two growing seasons, the seedlings had doubled their average initial height (average height: 106 cm in 2000, 203 cm in 2002, and 224 cm in 2003). Three growing seasons after outplanting some seedlings reach almost 4 m tall and were competing with yellow-poplar sprouts and seedlings.

Plantings of the Connecticut-grown seedlings in Connecticut incurred little mortality. Competition was restricted with the use of herbicides and hand-cutting, and the trees were protected from herbivory with plastic mesh tree shelters. The seedlings (from hand-pollinated crosses) were second-backcross Japanese (*Castanea crenata* Sieb. & Zucc.) hybrids crossed with two different American Chestnuts, and first-backcross Chinese (*Castanea mollissima* Blume) hybrids crossed with the same two Americans. Growth was best in three forest clearcuts, and was poor in an old field. Japanese hybrids averaged 258 and 225 cm height after three seasons in clearcuts and Chinese hybrids averaged 216 and 227 cm. In the old field Japanese hybrids averaged 213 and 119 cm and Chinese hybrids averaged 155 and 188 cm after three seasons.

There were differences in seedling growth across nitrogen content in two planting locations indicating chestnut's ability to respond to soil differences in both Connecticut and Kentucky. Chestnut is often considered a species that can grow across a wide range of nutrient conditions given its historical range.

Delineating nutritional factors that influence chestnut growth will provide better information for site selection in chestnut restoration and will allow for appropriate fertilization of seedlings to augment growth. Further investigations are needed into different soil conditions and nutrient amendments to determine their interactions with initial seedling growth.

ADDITIONAL CHALLENGES AFTER RESTORATION

Production of blight-resistant, timber-type chestnuts is an important milestone in the restoration of this genus to eastern North American forests. Resistance to chestnut blight disease is, however, only the first step toward restoration. There will probably be other challenges to chestnut throughout its life cycle from both native and exotic organisms. Indigenous pests such as the two-lined chestnut borer could emerge as serious problems, depending on the density and vigor of the restored species. Chestnut blight disease is just one of the serious exotic problems that planted chestnuts will face. Below is a list of some of the most serious exotic pests that may affect the plantings.

Phytophthora cinnamomi

Historically, mortality of American chestnut and Allegheny and Ozark chinkapins (*Castanea pumila* Mill. and *C. ozarkensis* Ashe., respectively) from root rot disease caused by *Phytophthora cinnamomi* is second only to chestnut blight disease. As mentioned above, the disease entered the country in Georgia during the early 1800s and rapidly spread. By 1878, American chestnuts in North Carolina River basins were noted to be dying (Hough 1878). American chestnuts and chinkapins were essentially eliminated from wet, poorly-drained soils and soils with heavy clay content by the turn of the 20th century (Crandall et al. 1945). Site selection is often considered the most important factor in reducing losses due to *Phytophthora* (Agrios 1997; Campbell and Copeland 1954) and will be an important consideration in planting blight-resistant chestnut.

Chestnut Gall Wasp (*Dryocosmus kuriphilus* Yasumatsu)

This insect was accidentally imported into the United States on smuggled budwood of Japanese chestnut, (Payne et al. 1975). The pest lays eggs in vegetative and floral buds, and feeding by larvae forms galls. Branch dieback can occur, and severe infestations can cause mortality. The range of chestnut gall wasp is still expanding north and west in eastern North America.

Exotic Ambrosia Beetle (*Xylosandrus crassiusulus* Mot. and *Xylosandrus saxeseni* Blandford)

These introduced insects have been found to infest chestnut seedlings and grafts in field and forest plantings and can cause mortality (Oliver and Mannion 2001). Other ambrosia beetle species have been recently imported into the eastern United States and could potentially cause problems for juvenile and adult chestnuts (Campbell and Schlarbaum 2002).

European Gypsy Moth (*Lymantria dispar* L.)

European gypsy moth will feed on American chestnut, but it is not a preferred species.

Sudden Oak Death (*Phytophthora ramorum* Werres et al.)

Sudden Oak Death was first detected in California coastal forests in 1995 (Werres et al. 2001), and has since progressed into Oregon forests. The disease causes mortality in a number of hardwood species, including *Quercus* and *Lithocarpus*, which are in the same family (Fagaceae) as *Castanea*. *Phytophthora ramorum* has a wide host range and occurs on rhododendrons and other species grown by the nursery

industry. USDA APHIS did not initiate interstate restrictions on movement until 2002. Currently, it has been discovered in 14 other States including a number of eastern states on nursery stock exported from California and Oregon (F.T. Campbell, Pers. Comm., June 3, 2004). Tests have confirmed that European chestnut is susceptible to *Phytophthora ramorum* (Defra, 2004). Although this pathogen has been brought to eastern states on nursery stock, confirmation of infestation in natural or urban trees has yet to be reported. In addition to these species, the number of exotic pests is likely to increase in the forthcoming years due to trade agreements and treaties that fail to adequately protect the United States from new threats (Campbell and Schlarbaum 2002).

CONCLUSIONS

The above text has elucidated some of the factors that will contribute to or challenge the successful restoration of chestnut. Although it may be a number of years before significant numbers of blight-resistant seedlings are available, much information can be gathered by planting pure American chestnut or advanced generation hybrid chestnuts. Chestnut blight disease does not necessarily infect and kill young seedlings upon outplanting. Seedlings can maintain good health and growth for a number of years and thereby, contribute to the understanding of the silvics of the species. Plantings of advanced generation hybrid chestnuts on NPS lands can always be cut down after their study objectives are fulfilled. Concerns about pollen/seed contamination into existing American chestnut gene pools on NPS lands are negligible, as Asian germplasm is not competitive in eastern forests (Schlarbaum et al. 1994). Such plantings also can provide a better understanding of sites likely to have *Phytophthora cinnamomi*, which will be key to future successful chestnut plantings.

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EFFECTS OF PAST LAND USE AND INITIAL TREATMENT ON *CASTANEA DENTATA* SEEDLINGS

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Abstract: Efforts to impart blight-resistance to the American chestnut, *Castanea dentata,* have yielded strains with some ability to resist the disease. However, in addition to Chestnut blight, root rot caused by *Phytophthora* is a major impediment to the re-introduction of the chestnut. The degree of presence of *Phytophthora* has been associated with soil moisture and loss of forestland to agriculture. At Mammoth Cave National Park, the effects of ectomycorrhizal fungi treatments and anti-fungal treatments were tested on chestnut seedlings. In addition, the effects of placing seedlings in disturbed (*e.g.*, agricultural) or undisturbed areas were analyzed. No significant difference ($p=0.09$) in survivability was found between disturbed and undisturbed sites. In addition, seedlings in disturbed plots grew significantly more in height than undisturbed plots ($p=0.01$), though with no difference in diameter ($p=0.17$). The use of the fungicide Ridomil gold EC^{tm} in conjunction with greater soil preparation was found to increase survivability in disturbed plots ($p<0.0001$), though not in undisturbed plots ($p=0.76$). Ridomil did not have a significant effect on growth. The effect of mycorrhizal treatments on survivability was not significant when compared to control treatments ($p=0.91$).

INTRODUCTION

The American chestnut, *Castanea dentata*, was once an abundant canopy tree of Eastern hardwood forests. It was highly valued by both wildlife and humans for its nuts and quality timber. The chestnut was often 25 percent of the forest or more, and grew to be one of the largest trees in its ecosystem type at up to 100 ft tall and 5 to 10 ft in diameter (Ronderos 2000). Mammoth Cave National Park is on the western border of the chestnut's native range, although recent searches have found a number of young trees and root-sprouts throughout the Highland Rim region of Kentucky and Tennessee. During the summer of 2003 a researcher found over 1000 small American chestnut trees in the Big Woods, an old growth area within Mammoth Cave National Park (Mark Depoy, Mammoth Cave National Park (pers. com,). A review of surveyed deeds between 1931 and 1937 within the area of the current park found that chestnut was noted on 27% of the deeds and that chestnuts comprised 5.1% of corner trees (Rhoades 2002).

The demise of this stately tree began when a deadly fungus was introduced into the United States near the turn of the century. The fatal blight-causing fungus, *Cryphonectria parasitica,* was discovered in New York City in 1904, and was probably introduced on ornamental chestnut trees imported from Asia. With New York as the epicenter, the fungus spread rapidly throughout the eastern United States, and by the 1950s, nearly all of the American chestnut trees in the 9 million acres of their natural forest habitat had been killed (Ronderos 2000, Smith 2000). Only a few large chestnuts and some shrubby, blight-infested trees survived the initial epidemic. The blight only top-kills chestnuts, leaving the root system intact so that many trees re-sprout after the main stem dies. Although there are many of these sprouts left within the original range, they usually do not survive long enough or remain healthy enough to begin producing seed.

After the near loss of this keystone species, the search for blight-resistant trees began, and resistant parent trees were bred together in the hopes of generating even more blight-resistant offspring. Researchers and arborists also began back-crossing American chestnuts with completely resistant Asian species to create blight-resistant hybrids. Both hybrid and 100% American chestnut breeding programs have progressed significantly toward their goals, but there is not yet a genetically pure tree that is completely resistant to the chestnut blight. Many universities and natural areas programs have begun planting the more resistant trees that are available in an attempt to reintroduce *Castanea dentata* to eastern forests, but there are many difficulties associated with this process.

One of these difficulties is caused by another harmful fungus, *Phytophthora cinnamomi*, which was noted in trees even prior to the introduction of blight (Rhoades, 2003). It is thought to be associated with levels of soil moisture and with the clearing of forest land for agricultural use. Rhoades *et al.* (2003) found that chestnut seedling mortality was highest in wet, compacted soil such as would be common in areas highly disturbed by agriculture.

In some studies, ectomycorrhizal fungi treatment has been shown to protect against root rot. Anti-fungal treatments have also shown promise in preventing the disease (Marx and Davey 1969).

In the current study at Mammoth Cave National Park (MACA), 2,000 1-year-old 100% American chestnut seedlings from the American Chestnut Cooperators Foundation were planted in various locations within the Park. We tested these trees for their growth responses to application of additional mycorrhizal fungus, fungicide treatment, and control (no treatment). We also tested for the presence of *Phytophthora* fungus within these treatment groups and analyzed a potential correlation between *Phytophthora* and tree mortality.

In this study we hypothesized that the trees would have higher survival rates and more growth in undisturbed soils, and with either fungicide or mycorrhizal treatment. We also hypothesized that tree mortality would be associated with the presence of *Phytophthora*.

METHODS

Sites and Treatments

We selected 20 sites within Mammoth Cave National Park for these plantings (Table 1). Plots were placed on north- or northeast-facing slopes since evidence has shown this may aid the trees in resisting chestnut blight. The spring and fall freezing and thawing associated with south- to west-facing slopes accelerates the bark-splitting that increases the chance of fungal infection. In addition, the chosen sites were well-drained with a sandstone substrate and acidic soil type. All plots were placed in areas with relatively open understories and at least some canopy opening. We placed ten plots on soils which have historically experienced disturbance of the mycorrhizal layer (*i.e.*, agriculture), and ten plots in areas with no known history of soil disturbance (including the Big Woods area).

One hundred saplings from five different parentages were planted 2 m apart in a grid pattern within each of the 20 m x 20 m plots. Each tree was marked with a metal tag with a unique identification number that was associated with its plot, specific location within plot, and family designation. We measured the height in centimeters (cm) and root crown diameter in millimeters (mm) of each tree at planting. On the plot grid, each row of 10 trees began on the downhill (northeast) end of the plot and ran upward to the top (southwest) end of the plot. After planting, vented bottles containing cotton balls soaked in coyote urine were hung at the four corners of each plot to deter deer from browsing on trees.

Table 1. Plot number, soil type, planting date, and area of plantings.

Plot	Soil Type	Date Planted	Location Description
1	undisturbed	3/21/2003	Houchens Ferry
2	disturbed	3/25/2003	Houchens Ferry
3	disturbed	3/24/2003	Houchens Ferry
4	disturbed	3/25/2003	First Creek, East
5	disturbed	3/26/2003	First Creek, East
6	undisturbed	3/27/2003	First Creek, West
7	undisturbed	3/26/2003	First Creek, West
8	disturbed	3/28/2003	First Creek, West
9	disturbed	4/1/2003	First Creek, West
10	undisturbed	4/2/2003	First Creek, West
11	disturbed	4/2/2003	Blue Spring Hollow
12	undisturbed	4/3/2003	Blue Spring Hollow
13	undisturbed	4/4/2003	Cubby Cove
14	undisturbed	4/4/2003	Cubby Cove
15	undisturbed	4/7/2003	Big Woods
16	disturbed	4/8/2003	Big Woods
17	undisturbed	4/8/2003	Big Woods
18	disturbed	4/8/2003	Big Woods
19	disturbed	4/10/2003	Big Woods
20	undisturbed	4/11/2003	Blue Spring Hollow

Within each plot, we applied three different treatment types. One-third of the trees were root-dipped in an ecto-mycorrhizal gel and wrapped in wet paper for transportation to the planting sites. This gel was prepared by mixing 53 grams of DieHard^{tm} Ecto Root dip with 113 grams of Horta-Sorb Sm^{tm} water-absorbent gel and 18.93 L of water in a 5 gallon bucket.

The fungicide treatment group was treated by watering in approximately 325 ml of a solution of 1.3 ml of Ridomil gold EC^{tm} in 11.63 L of water at planting. In order to apply the fungicide we prepared a hole, broke up the soil, and watered the solution into the hole before planting the trees.

The control group was planted with no treatment, but roots of both control and Ridomil-treated trees were wrapped in wet paper to keep the roots from drying en route to the planting sites. For efficiency of planting, these two groups were planted using a dibble or planting bar method.

In order to keep treatments from mixing, treatment groups were arranged by row within the plot, with treatment rows chosen randomly. For example, row 1 may be treated with Ridomil (R), row 2 with mycorrhizae (M), and rows 3 and 4 control (C), etc. One row, also randomly assigned, was mixed (3R,

3M, 4C) so that the number of trees with each treatment would remain consistent throughout all of the plots.

At the end of the 2003 growing season we checked the trees for survival and re-measured the height and root crown diameter of each tree.

To test for presence of *Phytophthora* and mortality associated with it, we collected 188 trees from the plots in March 2004. We collected 141 dead trees and 47 live specimens and used an ELISA test to indicate presence or absence of fungus in the genus *Phytophthora*. The test kits were ordered from the Neogen company and the tests were performed with assistance from the Biotechnology Center at Western Kentucky University.

Statistical Design and Analysis

We used survival, height, and diameter of the trees to assess which treatment and plot type had the greatest success. We found the change in height and diameter by simply subtracting the height and diameter measurements at planting from the 2003 season end measurements.
We tested for correlation between height change and diameter change using a Pearson correlation matrix.

A Fisher's exact two-way (similar to chi-square) test was used to test for differences in the survival of seedlings in disturbed versus undisturbed plots.

A two-way Pearson's Chi-Square test was used to test for survival of seedlings by treatment type. One-way ANOVA were used to determine whether differences existed between the different treatments and agricultural versus non-agricultural sites.

RESULTS

We found an overall survival rate of 56% for the planted seedlings. Survivorship of *C. dentata* seedlings was variable from site to site (Figure 1), with visibly higher survival rates in Cubby Cove (sites 13 and 14) and the historically chestnut-rich area of the Big Woods (sites 15-19).

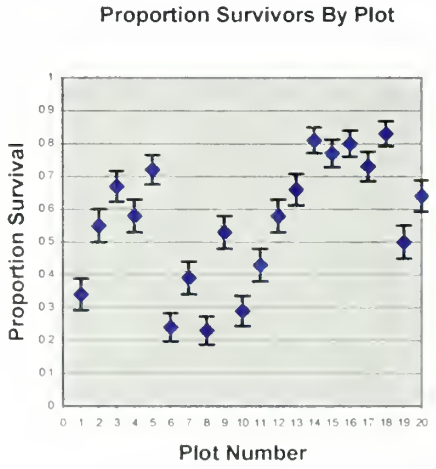

Figure 1. Survival rates for individual plots by plot number.

Percent survival by site type was 58.4% for disturbed sites and 54.5% for undisturbed sites (Table 2). Contrary to our initial hypothesis, we found no significant difference (p=0.087) in survival between disturbed and undisturbed sites using Fisher's exact test.

Table 2. Frequency of alive or dead trees at the disturbed and undisturbed sites. No significant difference in values, Fisher exact test (p=0.087).

	Disturbed	**Undisturbed**	**Total**
Alive	584	545	1129
Dead	416	455	871
Total	1000	1000	2000

To further test the differences between site types we calculated the amount of tree growth over the season and compared this between disturbed and undisturbed sites. Height difference and diameter difference were highly correlated ($r^2 = 0.695$) according to the Pearson correlation test, so because of that high correlation and the probability of error in the diameter measurements only height difference was used in the ANOVAs for difference between site types and treatments.

Using a one-way ANOVA for change in height, we compared disturbed to undisturbed sites and found that the disturbed sites had a significantly greater increase in plant height than undisturbed areas ($F=10.55$, $df=1$, $p<0.001$), as seen in Figure 2. Dead trees were measured as well, so desiccation and decay led to some negative change in height for both groups. This result also contrasted sharply with our initial hypotheses.

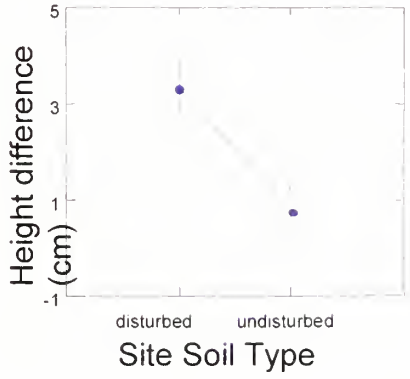

Figure 2. Height difference from planting to the end of the first growing season on disturbed (D) and undisturbed (UD) soils (p=0.001).

After comparing the site types to each other we separated them and tested for differences between treatments within a site type. Table 3 shows the survival rate of trees for each of the three treatments on undisturbed sites. We found no significant difference ($p=0.933$) in proportion of surviving trees for any treatment group within the undisturbed plots, indicating that neither fungicide nor mycorrhizal fungus had a strong effect on the survival of these seedlings.

Table 4 shows the same data for the disturbed plots. The only treatment associated with a significant difference in survival was the Ridomil treatment group (highlighted) with $p<0.001$. There are several possible reasons for this result related to planting method and site characteristics, which are considered in the discussion.

Table 3. Frequency of alive or dead trees at the undisturbed sites versus the three treatments. No significant difference was found using the Pearson Chi-Square Test ($p=0.933$).

	Control	**Mycorrhizal**	**Ridomil**	**Total**
Alive	184	179	182	545
Dead	157	151	147	455
Total	341	330	329	1000

Table 4. Frequency of alive or dead trees at the disturbed sites versus treatment. A significant difference in the Ridomil treatment group ($p<0.001$) is observed using the Pearson Chi-Square Test.

	Control	**Mycorrhizal**	**Ridomil**	**Total**
Alive	173	166	245	584
Dead	165	164	87	416
Total	338	330	332	1000

The last analysis we ran tested for the presence of *Phytophthora* fungus. Using the 188 collected trees, we took root scrapings per the kit directions and found an overall infection rate of 25.5%. To examine whether or not the infections were actually causing tree mortality, we compared the infection rate among the dead trees (28.4%) to that of the live trees collected (17.0%). Interestingly, there was no significant difference between the two infection rates ($chi^2=1.36$, $df=1$, $p=0.24$), indicating that *Phytophthora* is present within the plots but is not a major cause or tree death. We also isolated the results for dead and live trees in disturbed plots, where more infection and mortality was expected, and found the same non-significant result ($chi^2=1.82$, $df=1$, $p=0.177$).

DISCUSSION

There were several challenges involved in this experiment which may have had an effect on tree growth in our plots. Sites were chosen for relatively open understories, but there was still some degree of competition from other forest species, particularly the fast-growing red maples (*Acer rubrum*) and yellow-poplars (*Liriodendron tulipifera*). Some of the planted seedlings also fell prey to light browsing by deer in a few plots and leaf damage from insects in most of the plots. One challenge that limits our ability to compare treatments is the planting method. In order to apply the Ridomil properly we had to loosen the soil and water in the chemical, while the other treatment groups were slot-planted to expedite the planting process. This differential treatment may explain a great deal concerning the apparent significance of the Ridomil treatment discussed below.

We initially hypothesized that the trees would grow better in undisturbed soil sites and would survive at a higher rate with the anti-fungal treatment, but these hypotheses were not supported by the data. We found that seedlings grew more in height at the disturbed sites and that the Ridomil treatment had a significant positive effect on those sites as well. This initially led us to believe that *Phytophthora* was more of an issue in disturbed plots and that the Ridomil was mitigating that problem. When we tested for fungal presence, however, we discovered that this was not the case, since there was no significant difference in infection rates between living and dead trees including those on disturbed soils. A more probable suggestion is that the significance of the Ridomil treatment is caused by the difference in planting methods. While loosening the soil may not make a noticeable difference in light, undisturbed soils, it will have a greater impact on trees if the soil in the plot is compacted by disturbance and has higher clay content.

Although the growth was greater on disturbed soils, there was no significant difference in survival rate between site types, showing that the trees in our experiment still grew in the undisturbed areas, but a slower rate than at disturbed sites. In the Big Woods (plots 15-19), we found our highest survival rates, and this is most likely because of the site quality. Although all of the sites were chosen with the same criteria, the Big Woods area is known for the historic presence of large chestnut trees and currently holds over 1,000 natural seedlings and stump-sprouts, so more study is needed to find out exactly why *Castanea dentata* is so prevalent in this area and not in others.

We will continue to collect data on these trees as they mature, and larger trends may be discovered in future years. From this initial set of data, we see that undisturbed sites are not necessarily better habitat for chestnut plantings and that a more involved planting method and an initial fungicide treatment will aid tree growth within the first year. We also note that although *Phytophthora* fungus is present within our plots, it is does not appear to be a major cause of tree mortality within the sites we measured. We plan to continue this research by testing the soils and leaf-litter from each plot to determine more specifically what site characteristics lead to higher survival and greater growth among seedlings.

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POTENTIAL EXTENT OF AMERICAN CHESTNUT RESTORATION WITHIN THE NATIONAL PARK SYSTEM

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Abstract: A total of 128 National Park Service units and affiliated areas totaling 1.9 million acres were identified as being located within or closely bordering historic American chestnut range. Fifty two parks had historical records of American chestnut; 35 of which were aware of living remnants within their boundaries. There is widespread interest among park managers in restoring American chestnut if a blight resistant tree was available that met park objectives, was ecologically sound, and was approved by NPS policy. The strength of the Park Service in contributing to a chestnut restoration program may lie more in numbers, distribution, variety, and miles of parks rather than in surface acreage alone. These factors make the National Park Service uniquely positioned to contribute towards a rangewide restoration effort through education, interpretation, research, evaluation, and demonstration of emerging blight control and resistance technologies.

Keywords: National Park Service / Park Units / American chestnut / Restoration Potential

INTRODUCTION

The National Park Service (NPS) is a bureau of the U.S. Department of Interior with responsibility to preserve natural and cultural resources within congressionally designated areas for the enjoyment, education, and inspiration of current and future generations. There are approximately 388 units within the National Park system that collectively contain more than 83 million acres. In addition, the NPS cooperates with numerous federal, state, tribal, and local governments, private organizations, and businesses to extend benefits of resource conservation and outdoor recreation to many other public areas throughout the United States.

In May 2004, the NPS sponsored a workshop in Asheville, NC to review the ecological significance of the American chestnut (*Castanea dentata*) to eastern forests, update the status of efforts to develop blight resistant trees, and explore opportunities and risks associated with NPS involvement in future chestnut restoration programs. The initial purpose of this paper was to determine the number and size of NPS units located within historic American chestnut range so as to estimate the maximum possible extent of NPS involvement in a chestnut restoration program. Subsequent to the workshop, additional information was collected from park units on managers' knowledge of chestnut occurrence and their attitudes towards restoration. This information can be used to develop strategies and options that best utilize the unique resources of the NPS in a cooperative chestnut restoration program while meeting mandates to protect and preserve all natural and cultural resources within the National Park system.

MATERIALS AND METHODS

Identification of Park Units

National Park units located within or closely bordering historic American chestnut range were identified by overlaying American chestnut coverage data with NPS unit boundaries or point locations using

ArcGIS ArcMap 9.1 (Environmental Systems Research Institute, Redlands, CA). Eastern U.S. state and county boundary layers were obtained from The National Atlas of the United States of America (http://nationalatlas.gov) and historic American chestnut coverage was obtained from Little's Range and FIA Importance Value Distribution Maps (USDA Forest Service, Northeastern Research Station, http://www.fs.fed.us/ne/ delaware/4153/global/littlefia/species_table.html).

A list of NPS units in the eastern U.S. was compiled from entries in NPS ParkNet (http://www.nps.gov), the NPS Owner's Manual (National Park Foundation 2002), and a National Park guidebook (Scott and Scott 2002). Parks were identified as individual units if they appeared on the list of "384 NPS Units" (File dbo_NPSDirectory_places.xls; NPS NR-GIS Metadata and Data Store; http://science.nature.nps.gov/nrdata/) and they were not part of another NPS administrative group. Parks within an administrative group were listed individually if all units in the group were included in the list of 384 parks, but were lumped with their administrative group if the group reported acreage from holdings not included in the 384 park list. For example, Roosevelt-Vanderbilt National Historic Site parks (ROVA) were treated individually, whereas National Capital Central parks (NACC) were treated as a single administrative unit.

Park boundary coverage and additional unit information was obtained from the NPS NR-GIS Metadata and Data Store (http://science.nature.nps.gov/nrdata/). Appalachian Trail coverage was obtained from the Appalachian Trail Conservancy (http://www.appalachiantrail.org). All data layer projections were defined as NAD 1983 UTM Zone 17N and park units in chestnut range were extracted using ArcToolbox. Park acreage was obtained either from FY2004 data reported for each unit on ParkNet (http://www.nps.gov/parks.html; see "Facts: Acreage" under each unit's web page) or from boundary calculations of shape files obtained from the NPS NR-GIS Metadata and Data Store (http://science.nature. nps.gov/nrdata/).

Questionnaire

A questionnaire requesting input on the following six questions was sent to each park unit identified as being within or closely bordering historic chestnut range (choices are in parentheses):

1) Were American chestnuts ever present in your park? (Yes, No, Unknown)
2) Are there any living American chestnuts in your park today? (Yes, No, Unknown)
3) Would your park be interested in restoring American chestnuts if a blight resistant form was available? (Yes, No, Unknown)
4) If so, for what purpose? (Ecological Restoration = large scale reforestation; Demonstration = small plots for research purposes; Education = individual trees for historic reference or public education)
5) If so, how many acres or how many trees would you anticipate at full restoration?
6) If available, and within NPS policy guidelines, would the park utilize any or all of the following products? (Pure American chestnut selected for blight resistance; American x Chinese chestnut hybridized for resistance but retaining American form; Genetically Modified American chestnut with genes inserted for blight resistance from a different plant)

Questionnaire recipients were identified through a search for managerial or natural resource staff through the NPS People and Places Directory (http://data2.itc.nps.gov/npsdirectory/). Affiliated areas and trails were not sent a questionnaire nor were acreages determined for those units.

RESULTS

A total of 128 National Park units and affiliated areas were identified as being within or closely bordering historic American chestnut range (Figure 1). These included 91 parks totaling 1,729,730 acres found within chestnut range, 21 parks totaling 130,686 acres bordering chestnut range, and 16 affiliated areas

and trails with undetermined acreage wholly or partially within chestnut range (Table 1). Represented within this group were 53 Historical Parks and Sites, 19 Military Parks and Battlefields, 12 National and Natural Parks, 11 Memorials and Monuments, 8 Rivers, 7 Heritage Areas, 6 Trails, 5 Recreation Areas, 3 Parkways, 2 Preserves, and 2 Seashores. Park units were found within historic chestnut range in every state except Delaware, Indiana, and Florida. Excluding affiliated areas, parks ranged in size from 1 to 521,752 acres (mean = 16,611 acres; median = 700 acres). The largest unit, Great Smoky Mountains National Park, accounted for almost one third of the total acreage found within historic American chestnut range.

Questionnaires were sent to 104 of the 128 identified units, of which 81 parks (78%) responded; 63 parks within chestnut range and 18 parks bordering chestnut range (Table 1). Of the 63 parks located within chestnut range, 44 parks (70%) had historical records of American chestnuts on park land, of which 32 parks (51%) were aware of remnant trees currently surviving on park grounds. Of the 18 parks bordering chestnut range, 8 parks (44%) had historical records of chestnut, of which 3 parks (17%) were aware of present day specimens. A total of 53 parks indicated interest in restoring American chestnut, while 11 were not interested and 17 were unsure at this time. Of those parks expressing an interest, 31 would restore for ecological restoration of forests, 38 for demonstration and research purposes, and 34 for public education (parks could respond to more than one category). An additional 7 parks expressed an interest in using American chestnuts within historical or cultural landscape restoration, which was a category omitted from the questionnaire but should have been included.

Forty parks identified a total of 79,441 acres for potential chestnut restoration and an additional possible need for 1,375 individual trees. These acres represent a total possible land area where American chestnuts could be incorporated into the forest or landscape, and no attempt was made to determine if this land was actually suitable for chestnuts or the tree density the land would support. Of the 63 parks that expressed an opinion on product type for potential restoration, all would accept a pure American selected for blight resistance. If a pure American was unavailable, 35 parks (56%) would consider using an American x Chinese hybrid and 35 parks (56%) would consider using an American chestnut with genes inserted for blight resistance from a different plant, assuming that use of the later two products were approved by NPS policy. Comments from individual parks on historical and present chestnut occurrence and attitudes towards restoration are listed in Table 1. Some comments were paraphrased for brevity.

DISCUSSION

Recent advances in hybridization and genetic engineering have opened the possibility of controlling or managing the detrimental effects of chestnut blight in North America within the next several decades. Although these advances hold great promise to restore the American chestnut to its' former ecological role in eastern forests, the risks and benefits associated with using these technologies are poorly understood. Three paths are currently available to the National Park Service: 1) the NPS can chose not to be involved in chestnut restoration at this time and wait for other federal and private organizations to develop blight control technologies which the parks can implement after full testing; 2) the NPS can allow individual parks to adopt untested chestnut restoration technologies as they become available according to the level of risk each park is willing to assume, or; 3) the NPS can take an active, coordinated role in chestnut restoration by utilizing its unique resources to help other organizations and agencies develop and test new blight control technologies. Which direction the NPS chooses may well affect the ultimate success of returning this species to its native range during the next century.

Response to this questionnaire indicates that a majority of park managers within historic chestnut range would like to restore chestnuts to their park landscape and would actively participate in a coordinated NPS restoration program. There was equal or greater interest in participating in a restoration program

through education and demonstration projects as there was for actual reforestation, and numerous parks expressed interest in incorporating American chestnuts into the cultural landscape. Although all managers would prefer using a pure American strain for restoration, there was no widespread objection to using an American x Chinese hybrid or a genetically modified tree if the product met park objectives, was ecologically sound, and was approved by NPS policy.

This study identified over 100 parks and affiliated areas in historic chestnut range with total management area of approximately 1.9 million acres. Many of these parks have both historic and contemporary records of American chestnuts within their boundaries. Although this area represents less than 1% of the 200 million acres that chestnuts once occupied in the eastern United States (see D.E. Davis, this proceedings for total historic acreage), these parks are widely distributed throughout all of historic chestnut range. Park lands may thus contain the full scope of habitat types once utilized by American chestnuts and remnant sprouts may contain the full array of remaining genetic variability of the species. The strength of the Park Service in contributing towards an American chestnut restoration program may thus lie more in numbers, diversity, and geographic distribution of parks than in total surface acreage available for reforestation. This makes the Park Service uniquely positioned to cooperate with other public and private organizations to assist in chestnut restoration through public education, interpretation, demonstration, research, and long-term product evaluation. In addition, much of the acreage under NPS management exists in trails, parkways, and river corridors that traverse virtually all historic chestnut range. These units would be ideally suited to establish locally adapted source populations of blight resistant chestnuts for pollination and seed distribution into surrounding areas.

The overall positive response to this questionnaire and enthusiasm for American chestnut restoration among park resource managers indicates the Park Service should move forward in a coordinated effort to develop a Service-wide restoration program. Possible steps include formalization of restoration policy including guidance on use of genetically engineered products in National Parks, creation of a committee or council to guide restoration efforts, identification of NPS research priorities and information needs, clarification of roles that specific parks could play in chestnut research and restoration, centralization of NEPA planning, cataloging of remnant chestnut resources on park lands for use in regionally adapted breeding programs, and establishment of formal relationships with universities, federal and state agencies, and private organizations interested in pursuing cooperative chestnut restoration programs. These steps would position the National Park Service to become a leader and showcase for American chestnut restoration, and could serve as a template for restoring other native trees facing similar ecological threats from invasive diseases and pests.

ACKNOWLEDGEMENTS

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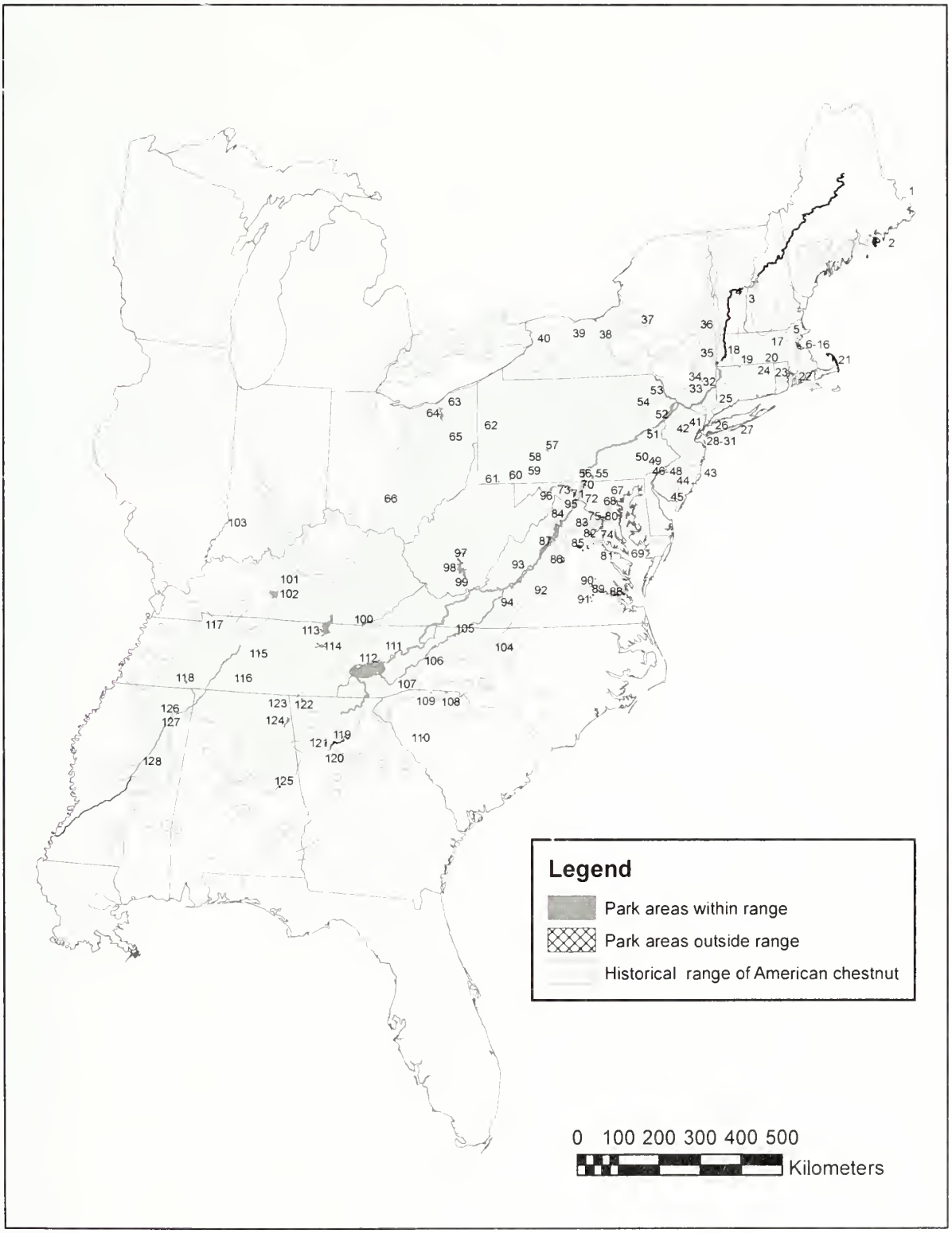
Figure 1. National Park Service units and affiliated areas located within historic range of American chestnut. Park numbers are identified in Table 1.[61]



[61] A full color, PDF version of this map is available at: http://chestnut.cas.psu.edu/nps.htm#lellis

Table 1. List of National Park units and affiliated areas within or immediately bordering historic American chestnut range [1, 2].

Map #	State	Park Name	Park Acres	Tree Type	Historically Present?	Potential Scope	Currently Present?	Would Park Restore?	Purpose	Comments
		PARK UNITS WITHIN CHESTNUT RANGE								
3	NH	Saint-Gaudens NHS	148	A,Hp, Gp	Y	75ac	Y	Y	R,D,E	Occasional stump sprouts. Saint-Gaudens NHS has been interested in this topic for a number of years and in 2002 hosted a public environmental education program entitled "Chestnuts in the 21st Century," featuring Dr. Sandra Anagnostakis of the Connecticut Agricultural Experiment Station, New Haven, CT. (Contact: Stephen Walasewicz, Natural Resource Manager)
6	MA	Lowell NHP	141							
9	MA	Minute Man NHP	971	A,H,G	Y	15-20ac	Y	Y	R,D,E	Minute Man NHP is about 970 acres and supports about 300 acres of upland forests. Remnant chestnut sprouts continue to inhabit perhaps 15-20 acres of the park. Ideally, it would be great to redistribute chestnuts throughout upland forests but re-establishing them on even 15-20 acres would be better than nothing. (Contact: Christopher Davis, Resource Management Specialist)
10	MA	Longfellow NHS	2							
12	MA	Boston African American NHS	1							
14	MA	John F. Kennedy NHS	1							
15	MA	Frederick Law Olmsted NHS	7							
19	MA	Springfield Armory NHS	55							
22	MA	New Bedford Whaling NHP	34							
23	RI	Roger Williams NMem	5							
25	CT	Weir Farm NHS	74	A,H	Y	15ac	Y	Y	R,E	(Contact: Greg Waters, Horticulturist)
26	NY	Sagamore Hill NHS	83	A,H,G	Y	U	Y	Y	D,E	There are remains of chestnuts that regularly put up root sprouts; however, these sprouts usually grow to about 10' tall and then die (probably from the fungus). We would have to look at the Cultural Landscape Plan & the Historic Plant Inventory to determine where chestnuts were located during the historic period to get a number. There is also the question of what to do with the 40-acre woods that were originally oak & chestnut, but have had successional growth of beech and oaks to replace the chestnuts. This is a great idea, but it sounds expensive. (Contact: Amy Verone, Park Curator)
27	NY	Fire Island NS	19,579							
28	NY NJ	Gateway NRA	26,607	A,H,G	U	1,500ac	U	Y	R,D,E	(Contact: Doug Adamo, Chief Division of Natural Resources)

Map #	State	Park Name	Park Acres	Tree Type	Historically Present?	Potential Scope	Currently Present?	Would Park Restore?	Purpose	Comments
29	NY	Governors Island NM	23							
30	NY	Manhattan Sites	10							Includes Castle Clinton NM, Federal Hall NMem, General Grant NMem, Hamilton Grange NMem, Saint Paul's Church NHS, and Theodore Roosevelt Birthplace NHS.
31	NY NJ	Statue of Liberty NM	58	A,H,G	N	5-10 trees	N	Y	E	Includes Ellis Island NM. Probably not on Liberty Island since we have a designed historic landscape. There might be a possibility for a few (5-10 trees) on Ellis Island though in the non-historic landscape portion of the island Would be glad to support effort within NPS policy guidelines. (Contact: Al Farrugio, Horticulturist)
32	NY	Eleanor Roosevelt NHS	181	A,H	Y	U	Y	Y	D,E	Comments included with Home of FDR NHS. (Contact: Dave Hayes, Natural Resource Program Manager)
33	NY	Home of Franklin D Roosevelt NHS	255	A,H	Y	U	Y	Y	D,E	We would not consider GMO as would jeopardize potential for FSC certification of Roosevelt tree plantation which we are considering at this time. Hybrid would be considered for cultural landscapes only, not native hardwood forest. (Contact: Dave Hayes, Natural Resource Program Manager)
34	NY	Vanderbilt Mansion NHS	212	A,H	Y	U	Y	Y	D,E	Comments included with Home of FDR NHS. (Contact Dave Hayes, Natural Resource Program Manager)
35	NY	Martin Van Buren NHS	40		U		N	N		According to records, the primary trees were white pine, basswood, and black locust. There is no evidence of any chestnuts. Being a cultural landscape, we are sticking to the trees that are recorded on the site when Martin Van Buren was present. His estate was called Lindenwald for the linden/ basswood. The black locust was imported from the lower Midwest and had become one of the primary species. The farm contained apple and pear orchards, crops, hay, and/or pasture. The fence rows were cleaned regularly and there was no evidence of wood lots. Kinderhook Creek has some elms, willows, and sycamores. (Contact: Randy Ross, Facility Manager)
36	NY	Saratoga NHP	3,392	A,H,G	Y	100+ac	Y	Y	L	Yes, in the form of stump sprouts. They only get to about 1" dbh before succumbing. Would restore in important interpretive sites to better represent the 18th century forest. (Contact Chris Martin, Resource Program Manager)
37	NY	Fort Stanwix NM	16		U		N	N		On the park's approximately 16 acres the Fort takes up the majority of space. The rest was cleared of trees for artillery/ musket kill-zone. For street frontage some flora is planned, but no chestnuts are considered. (Contact. Michael Kusch, Chief Interpretation and Resource Management)
38	NY	Woman's Rights NHP	7							
40	NY	Theodore Roosevelt Inaugural NHS	1							
41	NJ	Edison NHS	21							
42	NJ	Morristown NHP	1,711	A,G	Y	300ac	Y	Y	R	(Contact: Robert Masson, Biologist)
46	PA	Edgar Allan Poe NHS	1							

Map #	State	Park Name	Park Acres	Tree Type	Historically Present?	Potential Scope	Currently Present?	Would Park Restore?	Purpose	Comments
47	PA	Independence NHP	45	A,H,G	Y	5 trees	N	Y	E	To my knowledge, we will have one modern hybrid when current landscape plans are carried out. We would be interested in planting some more to develop our arboretum; however we are an urban park with no forest (Contact: Susan Edens, Cultural Landscape Architect)
48	PA	Thaddeus Kosciuszko NMem	1							
49	PA	Valley Forge NHP	3,466	A,H,G	Y	1,000-1,500ac	N	Y	R	Would need more information. We are less interested in form, however, and more interested in filling the ecological niche chestnuts formerly occupied. (Contact: Deirdre Gibson, Chief of Planning and Resource Management)
50	PA	Hopewell Furnace NHS	848							
52	PA NJ	Delaware Water Gap NRA	68,714	A,H,G p	Y	4,800-17,400ac	Y	Y	R,D	A 1911 timber harvest report shows that 29% of the harvest was American chestnut; a 1750-1795 witness tree record reveals that 8% were American chestnut. The Park's main interests would be to (1) replant in areas of declining hemlock stands (up to 2,800 acres), (2) plant in areas where the park is demolishing old structures (> 100 sites), and (3) areas that open up in the forest canopy as a result of natural disturbance. Extrapolation of percentages of both the witness tree report and the 1911 timber harvest to Park restoration goals indicate total acreage could vary from 4,800 to 17,400 acres at full restoration (other literature places American chestnut to be as high as 40-60% of the canopy). It would make sense to create multiple small stands of chestnuts where openings occur and over time have them spread to other areas. (Contact: Larry Hilaire, Wildlife Biologist)
53	PA NY	Upper Delaware SRR	55,575	A,H,G	Y	31+ac	Y	Y	R,D,E	One that I know of and have seen. We only own 31 acres here ourselves, and not all of that would be suitable land, but from what I understand in communicating with local foresters there is some interest among private landowners in restoration plantings. We would want to consult with local foresters and property owners, but there is some interest and we would be supportive of this if it's ecologically sound. (Contact: Don Hamilton, Natural Resource Specialist)
54	PA	Steamtown NHS	62	U	U	1-2ac	U	U	E	If any were extant, it would likely be prior to 1855. After that date, major industrial development took place within what is now the park boundary. We are basically an historic industrial park. (Contact: Christopher Ahrens, Facility Management Specialist)
55	PA	Gettysburg NMP	5,990		U		U	N		Includes: Gettysburg NCem. Gettysburg NMP is charged with maintaining a July 1-3, 1863 setting. As far as we know, American chestnut was not a component of the forests during those 3 critical days. Advertisements in newspapers from the 19th century indicate the chestnut forests were in the more mountainous northern and western parts of the county. The location of sawmills in those same locations reinforces the hypothesis that this is where the good chestnut wood was available in abundance. No mention of chestnut woodlots or groves in any Cumberland township advertisements. Some local farmers had woodlots of oak-hickory on the farm proper, but also owned "mountain land" with "good chestnut stands" (a lot of these chestnut forest plots were actually in Franklin County). There is literally no evidence to

Map #	State	Park Name	Park Acres	Tree Type	Historically Present?	Potential Scope	Currently Present?	Would Park Restore?	Purpose	Comments
										support the historic presence of chestnut within the context of our nationally significant era of 1863-1938. (Contact: Zachary Bolitho, Natural Resource Specialist)
56	PA	Eisenhower NHS	690		U		U	N		Comments included with Gettysburg NMP. (Contact: Zachary Bolitho, Natural Resource Specialist)
57	PA	Allegheny Portage Railroad NHS	1,296	A,H,G	Y	U	U	U	R	Species was found and listed as rare in 1980-82 at Allegheny Portage Railroad NHS. Not found at Johnstown Flood N Mem. Any work would need to be within National Park Service guidelines. An environmental assessment/ impact statement would be required. Presently we have no sites needing to be reforested and work would further depend upon identification of suitable sites. (Contact: Kathy Penrod, Natural Resource Specialist)
58	PA	Johnstown Flood NM	164	A,H,G	U	U	N	U	R	Comments included with Allegheny Portage Railroad NHS. (Contact: Kathy Penrod, Natural Resource Specialist)
59	PA	Flight 93 NMem	1							
60	PA	Fort Necessity NB	903	A,G	Y	30ac	U	Y	R,D	This tree was the dominant species at the time of the Battle of Fort Necessity A project to restore the forested hillsides to the historic scene would benefit greatly from the inclusion of this species. The park would be willing to establish research plantings, but fencing would have to be done to protect from deer. Would need monitoring as to success of the plantings and continued resistance. At full restoration of the Great Meadows Cultural Landscape we would want to include resistant American chestnut in with a mix of other historical species (white oak, hickories, red oaks, sugar maple, etc.) and plant an area of approximately 30 acres. This is an exciting project and I hope that we can work together to bring this most valuable species back into our region and into our historic landscape. (Contact: Connie Ranson, Natural Resource Specialist)
61	PA	Friendship Hill NHS	675	A,G	Y	2-5ac	U	Y	D,E,L	Start small with 2-5 acres of trees that could be grown and used to replace other species lost from the cultural landscape (Contact Connie Ranson, Natural Resource Specialist)
63	OH	James A. Garfield NHS	8		N		N	N		Since we are trying to maintain the cultural landscape, we are not interested in planting any new American chestnuts as they were not historically present on the site. (Contact: Carol Spears, Site Manager)
64	OH	Cuyahoga Valley NP	32,861	U	Y	U	Y	U	U	Present, but not fruiting. NPS DRAFT Director's Order 77-5: Genetically Modified Organisms is currently out for comment. Once finalized should provide guidance concerning use of GMOs. (Contact: Meg Plona, Biologist)
65	OH	First Ladies NHS	1		N		N	N		Comments included with James A. Garfield NHS. (Contact: Carol Spears, Site Manager)
66	OH	Hopewell Culture NHP	1,170	A,H,G	U	30-50ac	N	Y	R	Historical presence unknown. Records exist for Ross County; however this area borders the western edge of chestnuts. If possible, planting within existing woodlots would help increase the diversity of our woodlots. We also have a few areas open for tree planting, and would be interested in having chestnut in the mix. (Contact: Myra Vick, Biologist)

Map #	State	Park Name	Park Acres	Tree Type	Historically Present?	Potential Scope	Currently Present?	Would Park Restore?	Purpose	Comments
67	MD	Hampton NHS	62	A	U	U	N	U	L	Comments included with Fort McHenry NM&HS. (Contact: Paul Bitzel, Horticulturist)
68	MD	Fort McHenry NMHS	43	A	U	12ac	N	U	L	Likely present, as many black walnut, hickory, and Chinese chestnut are present in the park. No documentation has been found or research completed to know for sure. Park would be interested in restoring if it can be determined that American chestnut was grown here. (Contact: Paul Bitzel, Horticulturist)
70	MD	Catoctin Mountain Park	5,810	A,Hp	Y	U	Y	Y	R,D,E	(Contact: James Voight, Resource Manager)
71	MD	Antietam NB	3,252	A,G	Y	U	N	Y	D	Includes Antietam NCem. (Contact: Joe Calzarette, Natural Resources Program Manager)
72	MD	Monocacy NB	1,647	A,H,G	U	200ac	U	Y	D	A 12-15' sprout may have existed in the late 1990's but present location is uncertain. We would be interested in some limited "demonstration" type restoration if it were available. I think the "small plot" idea would be best for us. We have a couple of contiguous forested areas that are about 100, 40, 40, and 20 acres in size that we could establish plots. (Contact: Andrew Banasik, Natural Resource Manager)
73	MD DC VA	Chesapeake & Ohio Canal NHP	19,586	A	U	1-2ac	U	U	D	(Contact: Marie Sauter, Natural Resources Management Specialist)
75	DC	National Capital Parks - Central	950							Includes Constitution Gardens Park, Fords Theatre NHS, FDR Memorial, Korean War Veterans Memorial, Lincoln Memorial, National Mall Park, National WWII Memorial, Pennsylvania Ave NHS, Thomas Jefferson Memorial, Vietnam Veterans Memorial, and Washington Monument.
76	DC MD	National Capital Parks – East	9,846	A,G	Y	U	Y	Y	R,D,E	Includes Fort Washington Park, Greenbelt Park, Piscataway Park, Frederick Douglass NHS, and Mary McLeod Bethune Council House NHS. Historically, what would be the density? Park may want to do some sites, but not others. What would be impacts to the existing forest structure if/when this species is reintroduced? Would the reintroduced species replace oaks as Sudden Oak Death syndrome takes hold here in the east? (Contact: Susan Rudy, Natural Resources Program Manager)
77	DC	Rock Creek Park	2,394	A	Y	few acres	Y	Y	D,E	Widely scattered suckers from the location of old decaying chestnut stumps or root stock. The existing small stems are uncommon in the park. Initially a few selected trees involving a few selected sites totaling only a few acres. Too many unknowns to predict the number of trees or acres that would be involved in full restoration. There is some concern on our part about changing the species makeup of the park woodlands. If blight resistant chestnuts are planted in the park and once again become the dominant tree, as was the case historically, the current dominant species like beech, oak, and poplar could be replaced. Are we managing our park for historic conditions or to maintain the current natural processes that are taking place? We would favor restoration efforts in isolated, small areas. The Park enthusiastically supports restoration efforts for the American chestnut and would assist with field research if possible. (Contact: Ken Ferebee, Natural Resource Management Specialist)

Map #	State	Park Name	Park Acres	Tree Type	Historically Present?	Potential Scope	Currently Present?	Would Park Restore?	Purpose	Comments
78	DC	President's Park (White House)	18							
79	VA MD DC	George Washington Memorial Parkway	7,210	A	Y	U	Y	U	R	Includes Arlington House NMem, Clara Barton NHS, LBJ Memorial Grove, and Theodore Roosevelt Island NMem Depends how close it is to the original genotype. If it is the same we would be interested. (Contact: Brent Steury, Natural Resources Program Manager)
80	VA	Wolf Trap NP for the Performing Arts	130							
83	VA	Manassas NBP	5,073	A,G	Y	U	U	Y	R	None found in last plant survey. We have Chinese chestnut in the Park, *Castanea mollisssisima*. (Contact: Bryan Gorsira, Natural Resource Program Manager)
84	VA	Cedar Creek & Belle Grove NHP	3,593							
87	VA	Shenandoah NP	199,045	A,Hp, Gp	Y	40,000ac	Y	Y	R,E	We estimate this to be approximately 40,000 acres, or 20% of the park. This estimate does not assume that the land would be restored to pure chestnut, but rather that chestnut trees have been included in the mixture of forest trees present. We anticipate that the majority of this acreage would be in backcountry areas impacted by forest-killing insects and disease; however some acreage would also be in developed areas where replacement trees were needed. GMO and hybrid may also be possibilities, depending on park service policy, tree availability, and known and perceived risks with these options. (Contact. Wendy Cass, Botanist)
88	VA	Colonial NHP	8,677							Includes Jamestown NHS, Yorktown Battlefield, and Yorktown NCem.
92	VA	Appomattox Court House NHP	1,774	U	Y	U	Y	Y	D	More information would be needed to make a decision on which product to use. (Contact: Brian Eick, Natural Resource Manager)
93	VA	Appalachian NST	227,001	A,Hp	Y	U	Y	Y	D	(Note: Map symbol displayed in VA, but trail traverses historic chestnut range in ME, NH, VT, MA, CT, NY, NJ, PA, MD, WV, VA, TN, NC, and GA) We might be interested in large scale restoration, but with 14 states to cover it would be too costly. We have more than 1600 occurrences of 300 rare, threatened, and endangered species that we are trying to protect I presume it would be hundreds of thousands or millions of trees that would be needed for full restoration. Our Appalachian Trail corridor acreage is 270,000 acres. I cannot say how much of that acreage would be chestnut habitat. Probably the most important result of the chestnut conference in Asheville was finding that a through-hiker of the Trail had documented all chestnuts along the AT from Georgia to Maine. I believe the number of chestnuts documented was 5,000. We spoke after the Asheville conference with regard to publishing an article on chestnuts along the Appalachian Trail. (Contact: Kent Schwarzkopf, Natural Resource Specialist)
94	VA	Booker T Washington NM	239	A	U	5ac	N	Y	D,E	No scientific documentation, however, Booker T. Washington mentions chestnut trees, nuts, and burrs in his writings about his early life here on this

Map #	State	Park Name	Park Acres	Tree Type	Historically Present?	Potential Scope	Currently Present?	Would Park Restore?	Purpose	Comments
										farm. My guess is that there probably were chestnut trees here in the 19th and possibly early 20th centuries. We would need to perform a site inspection to find out what suitable acreage might be available for small plots and consult with cultural landscape representatives to find out how many trees we could place and where. Best estimate is <5 acres of plots and 15-20 individuals. (Contact: Timbo Sims, Park Ranger)
95	WV	Harpers Ferry NHP	2,504	A	Y	2,500ac	Y	U	R	Yes, but they are in very poor condition. The park may be interested but further discussion is needed on the scope of a restoration and the factors that need to be considered. 80% of the park is forested (approximately 2,500 acres). If this project is undertaken, it should be coordinated and monitored by our regional natural resource stewardship office. That office has the professional expertise to evaluate this project. (Contact: Bill Hebb, Natural Resource Manager)
97	WV	Gauley River NRA	11,507	A,H,G	Y	U	Y	Y	D	Comments included with New River Gorge NR. (Contact: John Perez, Biologist)
98	WV	New River Gorge NR	72,189	A,H,G	Y	50ac	Y	Y	D	Depends on available funds, staffing, and compliance requirements, but possibly 50 acres. We have persistent chestnut sprouts in all three parks (NERI, GARI, BLUE), and would be interested in a reintroduction program. (Contact: John Perez, Biologist)
99	WV	Bluestone NSR	4,310	A,H,G	Y	U	Y	Y	D	Comments included with New River Gorge NR. (Contact: John Perez, Biologist)
100	KY	Cumberland Gap NHP	20,508	A	Y	14,000ac	Y	Y	R,D	(Contact: Ries Collier, Chief of Resource Management)
101	KY	Abraham Lincoln Birthplace NHS	345	A,H,G	Y	3ac	N	Y	D	No original chestnuts, only the new ones we have planted over the last two years. We began a chestnut restoration project through Mammoth Cave NP two years ago – so far we have replanted about 400 trees. Purpose of the project is demonstration. (Contact: Sandy Brue, Chief of Interpretation and Natural Resource Management)
102	KY	Mammoth Cave NP	52,830							
104	NC	Guilford Courthouse NMP	229	A,G	Y	300 trees	N	Y	R,E,L	(Contact: Steven Ware, Chief Ranger)
105	NC VA	Blue Ridge Parkway	93,390	A,H,G	Y	<10ac	Y	Y	D,E	It seems unlikely that American chestnut can be restored to its former glory – even with a blight resistant variety. We do not have sufficient data such as site locations or stand density to guide restoration. I suspect the funding would never be in place to undertake restoration at a landscape scale. Given that, I think the Blue Ridge Parkway could work to establish small colonies of blight resistant chestnut in appropriate habitats. Seed from these colonies could disperse via natural mechanisms to establish additional populations. I think a discussion about how to restore and the goals of restoration would be needed once a blight resistant form is developed. (Contact: Chris Ulrey, Plant Ecologist)
107	NC	Carl Sandburg Home NHS	264	A,H	Y	50 trees	Y	Y	D	(Contact: Irene Van Hoff, Forestry Technician)

Map #	State	Park Name	Park Acres	Tree Type	Historically Present?	Potential Scope	Currently Present?	Would Park Restore?	Purpose	Comments
108	SC	Kings Mountain NMP	3,945	A,H,G	Y	100+ ac	U	Y	D,E,L	This is of course merely a cursory interest at this point. Actual decisions that would lead to obligations would have to be made in the future and would be dependent upon financial feasibility, up-to-date species integration data, and relevant NPS policies. We look forward to hearing more information on the subject. (Contact: Chris Revels, Chief Ranger)
111	TN	Andrew Johnson NHS	17		U		N	N		ANJO has no forest areas within the site It is a 100% cultural manicured landscape with a tree plan for the National Cemetery and Presidential Home sites. No chestnuts are known to have existed during the President's lifetime. (Contact: Mark Corey, Superintendent)
112	TN NC	Great Smoky Mountains NP	521,752	A,H	Y	U	Y	Y	R,D,E	Large scale ecological restoration would not be attempted until research indicates it is feasible and ecologically sound The park is 800 square miles and chestnut was originally a part of nearly every forest type. (Contact: Kristine Johnson, Supervisory Forester)
113	TN KY	Big South Fork NR&RA	125,310	A,H	Y	25ac	Y	Y	R,D	Initially interested in demonstration, but potentially moving into ecological restoration at some point. Use of hybridized stock would be considered, but more information concerning tree properties is needed before we would commit. What percent purity? What are life attributes, etc.? (Contact: Bryan Wender, Botanist)
114	TN	Obed WSR	4,732	A	Y	100ac	N	Y	R	None of the park staff have seen any Suitable habitat may be missing from the park. (Contact: Nancy Keohane, Resource Manager)
115	TN	Stones River NB	709	A	U	U	N	U	D,E	Includes Stones River NCem. It is unlikely that American chestnuts were ever prevalent here in the Central Basin of Tennessee. Our flora differs significantly from the Highland Rim and Cumberland Plateau that surround us. Our soils are considerably less acidic. As a consequence, we lack many of the tree species found in these physiographic regions. On the edges of the Central Basin where topography begins to change and in isolated pockets with more acidic soils, such as Indian and Scales Mountains, American chestnut may have been more prevalent. If further research reveals that the American chestnut was indeed a component of our forests, we would be interested in restoring this species to the park (Note: flora differences refer to the Inner Basin, not the entire Central Basin of TN.) (Contact. Terri Hogan, Ecologist)
117	TN	Fort Donelson NB	552							Includes Fort Donelson NCem.
118	TN	Shiloh NMP	5,060	A,H	Y	U	Y	Y	D	Includes Shiloh NCem. (Contact. Marcus Johnson, Resource Management Specialist)
119	GA	Chattahoochee River NRA	9,271	A,H,G	Y	10-300ac	Y	Y	R,E	American chestnuts were and still are present within Chattahoochee River NRA. We likely would not be able to provide vast acreage for restoration, however, from 10-300 acres would be possible While not vast, having geographic and environmental variability in the restoration sites may be advantageous; if so, then we may be able to contribute in a small way. If allowable under existing policies, we may utilize all three of the chestnut choices. (Contact: David Ek, Chief Science and Resource Management)

Map #	State	Park Name	Park Acres	Tree Type	Historically Present?	Potential Scope	Currently Present?	Would Park Restore?	Purpose	Comments
121	GA	Kennesaw Mountain NBP	2,884	A,H	Y	1,000 trees	N	Y	E	(Contact: Lloyd Morris, Chief Ranger)
122	GA	Chickamauga & Chattanooga NMP	9,038	A	U	U	N	U	L	Not a species listed in our Cultural Landscape Report to be used in reforestation projects. Only if the tree was originally here in 1863. CHCH does not have a Natural Resource person to manage this project. (Contact: Jim Szyjkowski, Cultural Resource Manager)
123	AL	Russell Cave NM	310							
124	AL	Little River Canyon NPre	13,633	A	Y	100ac	Y	Y	R,D	(Contact: Mary Shew, Resouree Management Specialist)
126	MS	Brices Cross Roads NBS	1							
127	MS	Tupelo NB	1							
128	MS AL TN	Natchez Trace Parkway	51,984	A	Y	U	Y	Y	R,E	(Contact: Bill Whitworth, Natural Resources Management Specialist)
	PARK UNITS BORDERING CHESTNUT RANGE									
1	ME	Saint Croix Island IHS	45		U		N	N		(Contact: Linda Gregory, Botanist)
2	ME	Acadia NP	46,856		N		N	N		American chestnuts were not present in the park according to herbarium records for Hancock county. Maybe one extant population near Bucksport (same county) reported by McMahon, Jacobson and Hyland Because the species is not historically known from Mount Desert Island (where Acadia is located) the park probably wouldn't introduce the tree. Acadia tries to maintain a policy of only planting genetically native species. (Contact: Geneva Chase, Botanist)
4	VT	Marsh-Billings-Rockefeller NHP	643	A,H	U	U	U	U	D,E	None detected in silvicultural inventories. (Contact: Christina Marts, Resouree Manager)
7	MA	Salem Maritime NHS	9		Y		Y	N		We have one chestnut here that was planted about 15 years ago to replace a chestnut that died from blight years earlier. I wasn't here when the tree was removed, but I did help plant this one, which is doing well. (Contact: Tim Thornhill, Facility Manager)
8	MA	Saugus Iron Works NHS	9	A,H,G	U	4-6 trees	N	Y	E	(Contact: Daniel Noon, Biologist)
11	MA	Boston NHP	43		N		N	N		The landscapes at Boston NHP are primarily designed, historic landscapes in an urban setting. American chestnut was not present within the historic period being presented so would not be part of the palette of plants used in the park. The Historic Grounds Report for the Navy Yard and the Cultural Landscape Reports for Bunker Hill and Dorchester Heights indicate they all had American elms, lindens, maples, and other assorted trees, but no reference to American chestnut, either historically or currently. (Contact: Gene Gabriel, [illegible])
13	MA	Boston Harbor Islands NRA	1,482							

Map #	State	Park Name	Park Acres	Tree Type	Historically Present?	Potential Scope	Currently Present?	Would Park Restore?	Purpose	Comments
16	MA	Adams NHP	24	U	U	U	N	U	U	(Contact: Marianne Peak, Superintendent)
21	MA	Cape Cod NS	43,608	A	Y	U	Y	Y	D,E	I have seen only a few trees; less than 5 that I have personally observed. They are scattered throughout the Seashore but I have no idea about whether they were planted and how long ago. (Contact: Stephen Smith, Plant Ecologist)
74	MD	Thomas Stone NHS	328	A,G	Y	U	N	Y	R	Comments included with George Washington Birthplace NM. (Contact: Rick Morawe, Chief Natural and Cultural Resources Management)
81	VA	George Washington Birthplace NM	662	A,Gp	Y	400ac	N	Y	R	Possibly up to 400 acres+ (includes GEWA and THST). I do not believe we would consider hybrids at this time. Prefer to hold out for American native as per NPS management and introduction of exotic species policies. (Contact: Rick Morawe, Chief Natural and Cultural Resources Management)
82	VA	Prince William Forest Park	19,377	A,G	Y	U	Y	U	D,E	(Contact: Jennifer Lee, Biologist)
85	VA	Fredericksburg & Spotsylvania NMP	8,374	A,G	U	U	U	Y	R,D	Includes Fredericksburg NCem. Not sure if we have any, as no systematic survey has been completed, but based on my own personal observations and people from our tree crew, we have never seen any in the park. (Contact: Gregg Kneipp, Natural Resources Manager)
89	VA	Richmond NBP	2,517	A	U	U	N	U	E	(Contact: Kristen Allen, Natural Resource Management Specialist)
90	VA	Maggie L. Walker NHS	1	A	U	U	N	U	E	Would require discussion with historians, etc. If approved within NPS Guidelines, we would be equally likely to use any product, but like the idea of a pure American the best to maintain our native genotype. Maggie Walker NHS is several city blocks worth of buildings, so it may not be a good restoration site. (Contact: Kristen Allen, Natural Resource Management Specialist)
91	VA	Petersburg NB	2,739							Includes Poplar Grove NCem.
103	IN	George Rogers Clark NHP	26	U	U	3-4 trees	N	U	U	Historic landscaping plan would need to be consulted. Our park is urban mostly manicured landscape. A few acres of the park are added to the original area and therefore would be outside of the landscaping plan. It may be possible to have a few plantings in this area. (Contact: Frank Doughman, Chief I&RM)
109	SC	Cowpens NB	842	U	Y	U	N	Y	R,D,E	We had expressed an interest in the introduction of the American chestnut back into the landscape at Cowpens and have had discussions with the folks at Great Smoky Mountains NP. Chestnuts were part of the historic landscape at Cowpens NB. (Contact: Patricia Ruff, Chief Park Ranger)

Map #	State	Park Name	Park Aeres	Tree Type	Historically Present?	Potential Scope	Currently Present?	Would Park Restore?	Purpose	Comments
110	SC	Ninety Six NHS	1,022	A,G	Y	1-2ac	N	Y	E	Education, because of the historic use of the tree in 18th century America. (Contact: Eric Williams, Chief Park Ranger/ Historian)
120	GA	Martin Luther King, Jr. NHS	39							
125	AL	Horseshoe Bend NMP	2,040	U	Y	300-600ac	N	Y	R	Writings by William Bartram described the lands near the park in the 1770's as "having an abundance of chestnut on the hills," and Benjamin Hawkins described a 1798 Indian village three miles upstream from the park as having chestnut growing on the ridges. The park would seek policy and scientific guidance before making a decision. (Contact: Mark Lewis, Superintendent)

AFFILIATED AREAS AND TRAILS

Map #	Park Name	State	
5	Essex NHA	MA	
17	Sudbury, Assabet, and Concord WSR	MA	
18	Westfield WSR	MA	
20	Blackstone River Valley NHC	MA RI	
24	Quinebaug & Shetucket Rivers Valley NHC	CT MA	
39	Erie Canal NHC	NY	
43	New Jersey Coastal HTR	NJ	
44	New Jersey Pinelands NRes	NJ	
45	Great Egg Harbor NWSR	NJ	
51	Delaware & Lehigh NHC	PA	
62	North Country NST	NY PA OH	
69	Chesapeake Bay Gateways Network	MD DC VA WV PA NY	
86	Green Springs NHLD	VA	
96	Potomac Heritage NST	DC MD PA VA	
106	Overmountain Victory NHT	VA TN NC SC	
116	Trail of Tears NHT	AL GA KY NC TN	

[1] HTR = Heritage Trail Route; IHS = International Historic Site; NB = National Battlefield; NBP = National Battlefield Park; NBS = National Battlefield Site; NCem = National Cemetery; NHA = National Heritage Area; NHC = National Heritage Corridor; NHLD = National Historic Landmark District; NHP = National Historical Park; NHS = National Historic Site; NHT = National Historic Trail, NM = National Monument, NMem = National Memorial; NMHS = National Monument & Historic Shrine; NMP = National Military Park; NP = National Park; NPre = National Preserve; NR = National River; NRA = National Recreation Area; NRes = National Reserve; NS = National Seashore; NSR = National Scenic River; NST = National Scenic Trail; SRR = Scenic & Recreational River; WSR = Wild & Scenic River.

[2] Y=Yes; N=No; U=Unknown; R=Restoration; D=Demonstration; E=Education; L=Landscape; A=American chestnut; H=Hybrid, G=Genetically Modified; p=possible.

Steiner, K. C. and Carlson, J. E, eds. 2006. Restoration of American Chestnut To Forest Lands - Proceedings of a Conference and Workshop. May 4-6, 2004, The North Carolina Arboretum. Natural Resources Report NPS/NCR/CUE/NRR - 2006/001, National Park Service. Washington, DC.

SUMMARY OF FACILITATED WORKSHOP ON

RESTORATION OF CHESTNUT TO NATIONAL PARK SYSTEM LANDS

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Part of the goal of this conference was to develop a common understanding among participants of directions for American chestnut restoration programs on lands managed by the National Park Service (NPS), the principal sponsor of the conference. To achieve that goal, the third day was devoted to a facilitated workshop on this topic. Specifically, participants performed a guided SWOT (strengths, weaknesses, opportunities, and threats) analysis of the merits of NPS participation in the restoration of American chestnut given available technologies (including genetic technologies), existing knowledge of the ecological context of chestnut restoration, and likely regulatory and social dimensions of pursuing restoration using technologies available now or in the foreseeable future.

Strengths and weaknesses are internal issues, in this case the advantages or disadvantages that NPS would have as a participant or player in chestnut restoration. For these two discussion points, the focus was the organization itself relative to its role in reintroducing the chestnut. Opportunities and threats were defined as existing or potential conditions, external to the NPS, that might influence decisions to participate in restoration of chestnut. Opportunities and threats could include economic, ecological, and social influences, including the current state of knowledge and technology regarding chestnut restoration. Depending upon context, the same item can be regarded as either an opportunity or a threat.

Each participant was assigned to one of four groups for preliminary discussion of issues. Forty-two persons participated in this exercise (Table 1). Each group met in turn to consider the strengths, weaknesses, opportunities, and threats of NPS participation in the restoration of the American chestnut, and all participants reassembled after each session to report group lists. Following this, the lists were simplified by combining similar items, and each participant was asked to cast three votes for the most important item(s) in each of the four SWOT categories (a participant could place one vote on three items or use two or all three votes for a single item). The results of the voting are shown in Tables 2 through 5 for items that received two or more votes.

NPS strengths related to the restoration of American chestnut (Table 2) are predominantly associated with the size and history of the agency and its archival resources, reputation and appeal to the general public, land ownership and tenure, and effectiveness at educating the public. The preservationist mission of the NPS is also seen as an advantage in this context, and the federal "red tape" surrounding the implementation of NEPA (National Environmental Policy Act) regulations is seen as a safeguard against mistakes. The diversity of parks and their missions, and the relative autonomy of individual parks, were highlighted repeatedly as unusual for a large federal agency and regarded as potential strengths.

But many of the strengths of NPS were also cited as weaknesses when viewed in another light or by different participants (Table 3). In fact, the last-cited strength – park autonomy and diversity of missions – received the most votes as a weakness because autonomy can lead to poor coordination and inconsistency in policy, practice, and priority. Not surprisingly, insufficient or inconsistent funding (both intramural and extramural) received a large number of votes as a weakness (when is this not true?). Most of the remaining votes went to the existence of policies and cultural attitudes that stand in the way of applying new (or old) technologies to chestnut restoration. There was a lot of support for the idea that the

lack of a long-term NPS plan or policy is an impediment to pursuing initiatives like chestnut restoration. Of course, the principal reason for the workshop was to help formulate plans and policy.

Chestnut restoration seemed to be viewed as a good thing by most participants (Table 4). The potential emergence of new technologies and blight-resistant varieties was seen as an opportunity that the NPS, given its unique and significant role as a major land steward within the American chestnut native range, should pursue. Chestnut restoration was seen as probably ecologically beneficial to the forests managed by NPS, but participation in restoration was also seen as beneficial to the agency itself through the engenderment of public support, the furtherance of useful partnerships outside the agency, the enhancement of educational programs, and (more obliquely) the opportunity to sharpen NPS policy by focusing on a model species.

Most threats emphasized by participants (Table 5) seemed to arise from what could be called "the prudent exercise of extreme caution" rather than from actual knowledge of risks. Participants focused on unknown ecosystem effects arising directly from chestnut restoration (whether using transformed or hybrid material), the possibility of failure due to breakdown of resistance or the emergence of new diseases, and the unknown consequences of the changes that have occurred in the forest over the past 70 years and may occur over the next century. Also cited was the possibility that NEPA and other legal and regulatory issues could lead to a quagmire before restoration could even begin.

Workshop participants appeared to believe that NPS should at least articulate policy and at best actively participate in the restoration of chestnut in view of the NPS mission and its ownership of significant tracts within the original chestnut habitat, and also the fact that technologies may soon be available to actually achieve this long-sought goal. Clear policy or at least policy guidelines would alleviate the principal NPS weakness identified by participants – an inconsistency in policies and priorities among parks. Surprisingly, a significant number of participants (influential in the vote tally) seemed less interested in the potential ecological benefits of restoring this keystone species than in the unknown risks associated with non-native genetic material and possible perturbation to forest ecosystems that are, if not stable, at least reasonably robust after recovering from the loss of chestnut. This outcome seems to reflect a "desire to understand everything before doing anything" as identified in Table 3. The consensus appeared to be that chestnut restoration is a "high-gain" pursuit. Whether it is a "low-risk, high-gain" or a "high-risk, high-gain" pursuit remains unresolved by this workshop. The other papers in these proceedings may help answer that question.

Table 1. Participants in the facilitated workshop (**M** indicates the designated moderator).

Group 1	Group 2	Group 3	Group 4
Kim Steiner, **M**	John Carlson, **M**	Paul Sisco, **M**	Tim Phelps, **M**
Bill Lellis	Jim Sherald	Ray Albright	John Karish
Jenny Beeler	Brian Carlstrom	Jennifer Lee	Kristen Allen
Larry Hilaire	John Perez	Jennifer Hewitt	Michele Webber
Becky Loncosky	James Voigt	Chris McNeilly	Tom Blount
Mark DePoy	Greg Eckert	Mary Willeford Bair	Kent Schwarzkopf
Paul Berrang	Joe James	Ries Collier	Matt Diskin
Tom Kubisiak	Fred Hebard	Scott Schlarbaum	Sharon Friedman
Phil Pritchard	Albert Meier	Songlin Fei	Benji Cornett
Bill Powell	Peter Gould	John Bellemore	Sara Fitzsimmons
Dave Loftis	Will McWilliams		

Table 2. "Strength" items relating to NPS involvement in chestnut restoration that received multiple votes from participants.

Votes	Description
14	The NPS has a great deal of ecological data and information regarding the natural resources of national parks.
13	The NPS has a great deal of appeal and goodwill within the American public.
9	The NPS manages a large number of parks throughout the natural range of American chestnut.
9	NPS land ownership is long-term.
7	The NPS does well at outreach and public education.
7	Much of what remains of the natural genetic diversity of American chestnut is represented within national park lands.
7	NEPA implementation in the NPS provides a deliberative mechanism for environmental decision-making.
5	The diversity of parks and their missions within the NPS offers a variety of venues by which restoration can be approached.
5	Restoration and control of exotic species (like chestnut blight) have broad public appeal.
4	NPS lands are refugia against commercial exploitation.
4	The relative autonomy of individual units creates opportunities for many approaches and experimentation.

Table 3. "Weakness" items relating to NPS involvement in chestnut restoration that received multiple votes from participants.

Votes	Description
20	Individual units tend to operate rather independently, with resulting inconsistency, poor coordination, and variation in policy and priorities.
13	Funding is inconsistent and budgets and staffs tend to be small.
8	Competitive funding is uncertain and tends to support only projects in the 1-3 year range.
8	The NPS has no clear long-term plan that addresses issues like chestnut restoration.
7	Existing policies may stand in the way.
6	Private, proprietary rights to blight-resistant chestnut material could be an issue with the NPS.
5	The NPS can have a preservationist mindset that slows action, especially the desire to understand everything before doing anything.
4	Deer control is usually inadequate within national parks, and this could be an impediment to planting trees.
4	There is a perception within the NPS that manipulation is against regulations or at least discouraged.
3	Our inability to define "natural" stands in the way of defining management goals.
3	The NPS has had a history of poor cooperation with the USFS.
2	Staff and expertise are lacking for large-scale, landscape restoration.
2	NPS planning tends to be lengthy and cumbersome.

Table 4. "Opportunity" items relating to NPS involvement in chestnut restoration that received multiple votes from participants.

Votes	Description
18	New technologies and blight-resistant chestnut seed to enable restoration may be available soon.
16	As a large landowner, NPS can be a major participant in large-scale restoration.
8	Public (e.g., USFS) and private (e.g., landowners) cooperators are available for this work and offer opportunities not only for leveraging resources but also extending partnerships.
7	Chestnut restoration can enhance ecological integrity and stability of parks.
7	Now is a good time and chestnut is a good species for developing a policy model for species restoration.
7	Participation in chestnut restoration would play on public enthusiasm and engender support for the NPS.
6	Chestnut was a keystone species, and its restoration would have secondary benefits.
4	The chestnut story is a good vehicle for education about natural resources, exotics, and many other issues.
3	There is plenty of time to act on this issue so we can do so with all due deliberation.
3	Chestnut could be a replacement for other declining species.
2	Chestnut has characteristics duplicated by no other tree species, and it may be more adaptive to possible future changes in the environment such as climate change and air pollution.
2	Chestnut restoration has a social as well as a biological dimension.
2	Chestnut restoration could be the environmental success story of the 21st century.

Table 5. "Threat" items relating to NPS involvement in chestnut restoration that received multiple votes from participants.

Votes	Description
21	Restoration could have unknown negative ecosystem effects such as the inadvertent spread of pests, displacement of species, and even displacement of native chestnut.
14	Some may object to the use of GMOs or backcross hybrid trees to restore a native species.
11	Resistance could break down in time as the fungal pathogen evolves.
10	NEPA and other legal and regulatory issues could lead to a quagmire.
7	Other diseases and pests could ruin our efforts to restore chestnut, and other exotic pests could prove to be as ecologically devastating as chestnut blight.
6	There is a possibility of failure following expensive and widely publicized efforts.
5	Environmental changes since the 1930's (sudden oak death, air pollution, climate change, etc.) may mean that the forests are not the same as what chestnut disappeared from.
4	NPS has no control over adjacent lands.
3	It is difficult to plan for a moving target (state of the forest ecosystem) 100 years from now.
3	Institutional fatigue can be disastrous to long-term projects.
2	Our scientific knowledge of this subject is incomplete.

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